AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 33 NUMBER 4 DECEMBER 2004

Editor-in-Chief
YETUNDE A. AKEN'OVA

Assistants Editor-in-Chief
A. O. OGUNNIYI
O. D. OLALEYE

ISSN 1116—4077

Afr. J. Med. med. Sci (2004) 33, 341-345

Bioequivalence study of three generic formulations of co-trimoxazole tablets in human urine

TO Ilupeju, FO Oladeinde, AA Olaniyi and MA Amosu*

*Department of Pharmaceutical Chemistry, University of Ibadan, Ibadan, Nigeria, and *The Living Rock Hospital, Old Bodija, Ibadan, Nigeria*

Summary

Many proprietary and generic formulations of co-trimoxazole tablets commercially marketed in Nigeria are mostly from Asian countries. Nigerians buy these products because of their cheaper prices but not confident with regards to therapeutic, quality, safety, and efficacy. Health professionals usually are cautious about drug product selection and substitution during prescription and dispensing. In this paper, the bioequivalence study of three multi-sourced (generic) co-trimoxazole tablets was carried out on the urine of twelve healthy volunteers. The reversed-phase high performance liquid chromatography was employed for the analysis. Sulphadoxine was used as internal standard. The limits of detection were 76.3 ng/mL for trimethoprim, and 61.9 ng/mL for sulphamethoxazole at 0.16 aufs. The linearity (n=5) for the calibration curve was of the order, 1.0000 for trimethoprim and 0.9998 for sulphamethoxazole; percentage recoveries for trimethoprim and sulphamethoxazole were 89.4 and 87.9% respectively. The relative bioavailabilities of the two generics to the innovator's product were 104.2% (trimethoprim) and 106.8% (sulphamethoxazole); 114.8% (trimethoprim) and 111.8% (sulphamethoxazole) for a product of reputable pharmaceutical company in Nigeria and Indian product respectively. In conclusion, the three generic formulations of co-trimoxazole tablets were biologically equivalent. Interchangeability of drugs in prescription and dispensing may be recommended in this situation.

Keywords: *Bioequivalence; co-trimoxazole; generic; interchangeability.*

Résumé

Plusieurs propriétaires pharmaceutiques et des formulations génériques des comprimés de cotrimoxazole en vente au Nigéria proviennent des pays asiatiques. Ces produits sont moins chér mais la question de la qualité, l'effect thérapeutique et l'efficacité restent incertaine. Les personels de santé font attention à propos de la selection durant la prescription et la substitution du médicament à la vente. L'étude de la bioéquivalence de 3 sources de cotrimoxazole étaient faite sur les urines de 12 volontaires sain par la phase renverse de la chromatographie liquide. La suphadoxine était utilisée comme reference interne. Les limites de détection étaient de 76.3 ng/ml pour le trimothroprime, 61.9ng/ml pour le sulfphaméthaxazole. La linéarite était en ordre , 1000 pour le triméthroprime et 0.9998 pour la sulfphamethaxazole. Le pourcentage de recollection de la triméthroprime et la sulfphaméthaxazole était de 89.4% et 87.9% respectivement. La disposition relative des 2 génériques au nouveau produit était de 104.2%(T) et 106.8(S) ; 114.8%(T) et 111.8(S) pour ce produit d'un industrie pharmaceutique reputée du Nigéria et d'Inde respectivement. En conclusion, les formulations géneriques du cotrimoxazole étaient biologiquement équivalent. L'interchangéabilite de médicament prescrit et dispense peut être recommendée dans ce cas.

Introduction

Co-trimoxazole is a broad-spectrum combination antibacterial agent containing sulphamethoxazole (SMZ) and trimethoprim (TMP) in the ratio 5:1. Although each of the drug components in the combination is bacteriostatic, co-trimoxazole is said to be either bacteriostatic or bactericidal [1]. The combination has been found useful [2,3,4] in the treatment of infections such as *pneumocystic carinii pneumonia, symptomatic shigella enteritis, systemic salmonella* infections (caused by ampicillin- or chloramphenicol-resistant organisms), complicated urinary tract infections, prostatitis, and many others. However, as with other antibacterial agents extensive use of co-trimoxazole may lead to the development of resistant organisms. Bioequivalence studies can be employed among other measures to reduce the problem of antimicrobial drug resistance.

A modified RP-HPLC (reduction of extraction solvent volume) was employed to compare the bioavailability of three multi-sourced co-trimoxazole tablets [5,6]. The brands were coded as Cs, innovator's product; Cb, a product of reputable pharmaceutical company in Nigeria

Correspondence: Dr. F.O Oladeinde, Center for Complementary & Alternative Medicine, Spencer 205B Morgan State, University, 1700 East Cold Spring Lane, Baltimore, MD 21251
E-mail: foladein@jewel.morgan.edu Tel: 443-885-1668, Fax: 443-885-8287

and Cv, an Indian product. All were pre-determined to be chemically equivalent. The chemical quantities of TMP and SMZ relative to those of Cs (innovator) were 99.8% and 102.4%, 101.2% and 106.6% for Cb and Cv respectively. The relative bioavailability of Cb or Cv to Cs was determined in the urine of healthy Nigerians after single oral administration to ascertain interchangeability in drug prescription.

Subjects, materials and methods

Subjects

Twelve healthy Nigerians (3 males and 9 females) volunteered to take part in the studies. They were 400 level students of the Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria. Their ages were between 18 and 29 years (mean 23.9±2.5) and their weight ranged from 45 to 75 kg (mean 59.4±8.5). All of the subjects were non-smokers, nor drank of alcohol. None took any medication regularly, nor had taken co-trimoxazole within one month prior to the study. The study design received the approval of the Ethical Committee of University of Ibadan and University College Hospital, Ibadan, Nigeria.

Drug administration and sample collection

The volunteers were divided into 3 groups of four. Each volunteer in a group took a particular brand of co-trimoxazole tablets at a particular round of the studies. A single oral dose of two tablets equivalent to 800 mg (SMZ) and 160mg (TMP) was administered to volunteers in a completely randomized balanced crossover manner for three consecutive rounds (one week for each round). Such design enabled each volunteer to act as his own control. The volunteers observed overnight fasts and remained fasted for a period of 2 hr after drug administration. No other medications or alcohol drinks were taken throughout the course of the studies. A wash out period of two days based on the half-lives and by comparison of cumulative amount excreted versus time plot for TMP or SMZ was observed between one round and the other to allow for maximum possible elimination of the drug before the next treatment. Total volume of urine was collected and noted from each volunteer at 24hr intervals before drug administration to serve as control and 5 consecutive days thereafter after dosing. Out of each of the samples, 30ml aliquots were set aside, preserved [7] with 0.5 M H_2SO_4 and kept frozen at about −10°C until analyzed. The extracted samples were subsequently transported frozen from the Faculty of Pharmacy, University of Ibadan to Roche (Nig) Ltd., Lagos, Nigeria for the HPLC analysis of TMP and SMZ. The total storage time was less than 2 months.

General experimental procedure

Roche Nig. Ltd., Lagos, Nigeria, supplied Trimethoprim, sulphamethoxazole and sulphadoxine. Chloroform (Fisher Chem Alert, USA); acetonitrile (Sigma-Aldrich, England); triethylammonium (Fisons Plc, England) and glacial acetic acid, sulphuric acid, hydrochloric acid, methanol, ethyl-acetate, potassium phosphate monobasic, sodium phosphate dibasic (all BDH Chemicals, Ltd., England) were utilized in the study.

Chromatographic conditions

The high performance liquid chromatography (HPLC) instrument used was a Waters Model 486 UV detector operated at 289 nm wavelength and sensitivity of 0.16 aufs. This was coupled to a Waters 510 pump operated at 1mL.min^{-1} flow rate and 2500 psi operating pressure; load/injection point of Rheodyne 77251 fitted with 20 µL loop and Waters 746 Data module recorder. The column was a stainless steel Nucleosil 100 C18, 7 µm, 250x4.0 mm (Macherey-Nogel AG) and the guard column: spheri-10-RP18, 10µm, 30x4.6 mm (Brownlee-Guard). The degassed vacuum filtered mobile phase was isocratic, made up of a mixture of triethylammonium acetate buffer (pH 4.4), methanol (HPLC grade), and acetonitrile (HPLC grade) in the ratio 75:20:5 respectively. The internal standard was sulphadoxine, IS-SDX.

Extraction procedure

To 1 mL of urine (as ° aqueous dilution) in a 12 mL glass extraction tube was added 3mL of phoshate buffer (0.2M, pH 6.2) and 6 mL extraction solvent (chloroform-ethylacetate, 75:25). The stoppered tube was vortex mixed for 1 minute and centrifuged at 3000 r.p.m. for 10 minutes. Then, 5 mL of the chloroform phase were transferred into another and evaporated to dryness under nitrogen at 50° C. The residue was reconstituted in 200 µL of mobile phase. After mixing for 20 seconds on a vortex mixer, 20 µL were injected into the column.

Recovery

The percentage recovery was determined by comparing the peak-ARE (amount remaining to be excreted) ratios of the extracted drugs with those obtained by direct injection of the same concentrations of the drugs. Three different concentrations were used to determine percentage recovery for each drug component viz: for TMP 1.5 and 20 µg/mL were used while 2, 50, and 150 µg/mL were utilized for SMZ. The limit of detection was calculated mathematically by estimating the concentration that corresponds to the noise/peak ratio of three standard deviations of the blank sample.

Specificity and retention times

The specificity of the analytical procedure was determined by injecting reference samples of TMP, SMZ and SDX (internal standard) individually, leading to retention time determination; and collectively, showing the degree of specificity of the method.

Data analysis

The concentration data obtained were analyzed assuming a one-compartment open model. ARE versus time plot (Sigma minus plot) was used to estimate the amount excreted at infinity and this parameter was utilized to compare the bioavailabilities of the tablet formulations. The biological half-life of the drug was calculated from sigma minus plot on semi-log paper, while elimination rate constant was determined from the slope of the curve. Relative bioavailability was estimated using the ratio of amount of drug excreted at infinity for the test drugs to the innovator's product. Statistical analysis was applied to the bioavailability parameter using student's t-test and Analysis of Variance (ANOVA) accepting $P<0.05$ as significant.

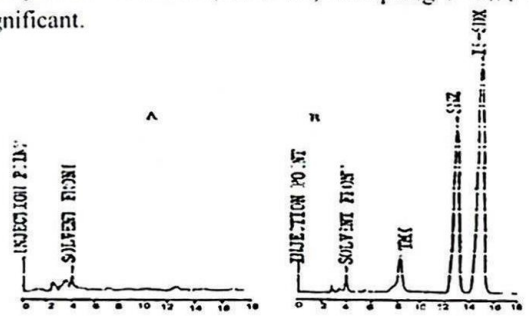

Fig. 1: HPLC chromatograms of (A) blank urine extract and (B) volunteers urine extract corresponding to 218 µg/mL sulphamethoxazole (SMZ) and 100 µg/mL internal standard-sulphadoxine (IS-SDX) with retention times of 8.36, 13.09 and 15.22 min. respectively.

Results

From the results, the pulse rates ranged from 64/min to 88/min (mean 76±10.5), blood pressure was between 90/60 and 120/70mm/Hg (mean 108.2±2.3/65.5±2.3). Fig. 1 shows the resultant HPLC chromatograms of the drug components in blank and test urine for the unchanged parent drugs (TMP and SMZ) as well as the IS-SDX. The retention times were 8.36 min (TMP), 13.09 min (SMZ), and 15.22 min (IS-SDX) eluting in that order. The limits of detection were 76.2ng/mL (TMP) and 61.9 ng/mL (SMZ) at 0.16 aufs.

The recoveries of the active components were 89.4% (TMP) and 87.9% (SMZ). The calibration curves for both drugs in urine were linear and of the order 1.0000 (Fig. 2a) and 0.9998 (Fig.2b) for TMP and SMZ respectively. An asymptotic graph was obtained on plotting the cumulative

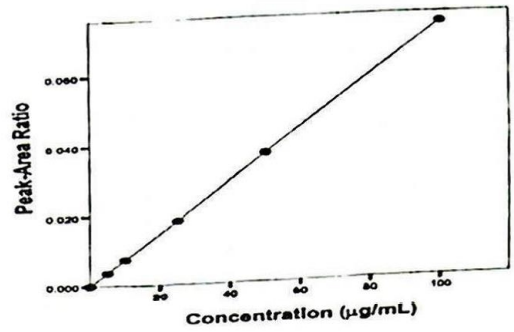

Fig. 2a: Calibration curve for trimethoprim

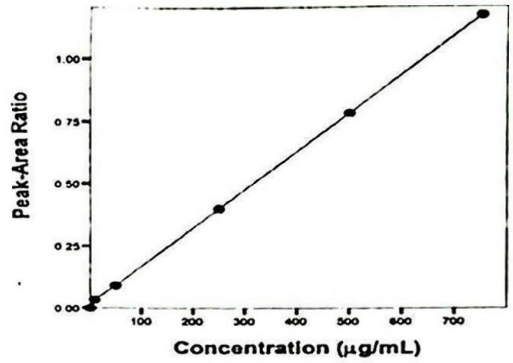

Fig. 2b: Calibration curve for sulphamethoxazole

amount excreted versus time for both trimethoprim (Fig.3a) and sulphamethoxazole (Fig. 3b). The half-lives (t^*) for TMP in Cs, Cb and Cv were 16.8, 19.2, 18.0 hr., and SMZ 12.0, 11.0 and 12.0 hr. respectively. Similarly, the values of the elimination rate constant, Kel were 0.0420, 0.0363 and 0.0390 hr^{-1} for TMP, while those for SMZ were 0.0583, 0.00612 and 0.0561 hr^{-1}. Bioavailability values relative to those of Cs of TMP and SMZ were 104.2% and 106.8%; 114.8% and 111.4% for Cb and Cv respectively. At 95% confidence limit, statistical analysis showed that the difference in values among the tablet formulations was not statistically significant ($P>0.05$).

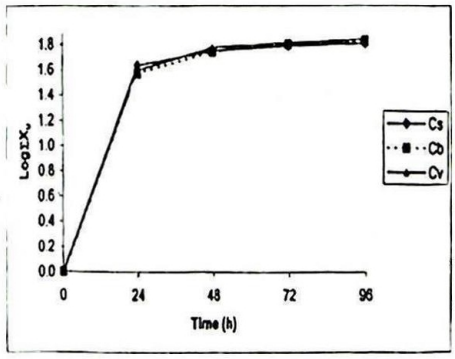

Fig. 3a: Comparison of cumulative amount excreted versus time plot for trimethoprim in Cs, Cb and Cv.

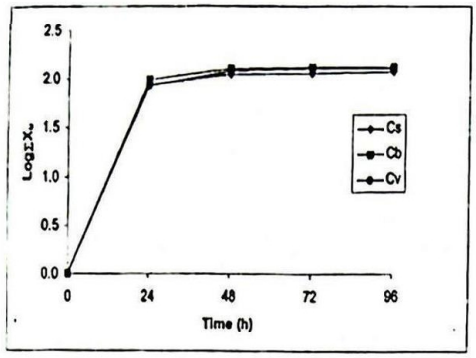

Fig. 3b: Comparison of cumulative amount excreted versus time plot for sulphamethoxazole in Cs, Cb and Cv.

Discussion

The resultant HPLC chromatograms (Fig.1) of the drug components in blank and test urine extracts demonstrated that the unchanged parent drugs (TMP and SMZ) as well as the IS-SDX showed good baseline resolution and separation.

Randomized cross-over design was observed to eliminate inter-subject variations as much as possible and also to make each subject stand as his own control [8]. Urine samples were used for analysis because the volunteers more readily gave them. Urine was the major route of elimination of the drugs. Plasma concentration determination was not carried out because the volunteers refused to give blood samples due to many mysteries (taboo) attached to blood donation and handling in Africa. The volunteers did not produce enough volume of saliva for analytical handling in order to correlate with urinary analysis. The urine integrity was maintained by preserving with 0.5M H_2SO_4 [7] in order to prevent degradation due to loss of carbon dioxide, which could increase the alkalinity of the urine and finally resulting in the precipitation of in organic phosphates.

The HPLC method of analysis used was simple and effective for the simultaneous determination of both TMP and SMZ contents of the combination drug product. The adapted method of analysis as described previously [5,6] was used with sulphadoxine as internal standard to ensure good resolution of peaks. An asymptotic graph obtained from the cumulative amount excreted versus time for both drugs signified as described previously [9] that the two days wash-out period allowed for maximum possible elimination of the drugs before the next treatment seemed adequate.

The mean percentages of the dose excreted of SMZ after 48hr were 14%, 16% and 15% for Cs, Cb and Cv respectively as against 18%, 31% and 3.4% obtained previously [9]. Similarly, TMP excreted from the three brands administered after 48 hr. were 35%, 35% and 37% respectively against 24.4% and 50-60% as described previously [5,8,10]. The t° for TMP and SMZ ranged from 16.8 to 19.2 hr. and 11 to 12 hr. (lag time included) for the various brands against 11-17 hr. and 9-12 hr. [8] respectively. All these variations could be due to difference in the analytical handling of procedures and individual rate of metabolism. Black Africans have been found to be slow metabolizers of the drug [11], also volunteers were encouraged to "force fluids" in order to improve urine collection and minimize variation [12]. This was reflected in the percentage of intact drug excreted by volunteers.

Statistical analysis employing student's t-test and Analysis of Variance (ANOVA) at 95% confidence limit showed that the difference in values among the tablet formulations were not statistically significant. Therefore, the multi-sourced co-trimoxazole tablets may still be interchanged during prescription and dispensing. The results have provided scientific justification to some multi-national pharmaceutical companies dealing exclusively with brand name products, sometimes having subsidiary firms, which manufacture drugs using the same raw materials and processes, but sell them only by generic names [13].

Acknowledgements

Our thanks go to Mr. Bola Keshinro of Roche Pharmaceuticals for his technical assistance.

References

1. Schild H.Q. Synthetic Compounds for Chemotherapy of Infections, In: Applied Pharmacology, 12th ed., Churchill Livingstones, London, 1980, 392-397.
2. Gilman A.G., Goodman L.S., Gilman A Trimethoprim-sulfamethoxazole. In: Goodman and Gilman's, The Pharmacological Basis of Therapeutics, 6th ed., Macmillan, London, 1980, 1116-1119.
3. Katzung B.G. Sulfonamides and Trimethoprim. In Basic and Clinical Pharmacology, 5th ed., Appleton and Lange, Connecticut, 1992, 661-666.
4. Rang H.D., Dale M.M., Ritter J.M. Antibacterial Agents In; Pharmacology, 3rd ed., Churchill Livingstone, London, 1995, 721-724.
5. Varoquaux O., Cordonnier P., Advenier C., Pays, M. Simultaneous HPLC Determination of trimethoprim, sulphamethoxazole and its N-4 Acetylmetabolite in Biological Fluids J.

Chromatogr., 274: 1983, 187-199.

6. Salem M.S., Alkaysi H.N. Gharaibeh A.M., Amari, F.F., Sallam, E., Shubair, M.S. Analysis and Bioequivalency study on two tablet formulations of co-trimoxazole .J Clin. Pharm. Ther., 13: 1993, 109-114.
7. Chamberlain J. Special problems with Biological fluids In: Analysis of drugs in Biological fluids; 1st ed., CRS Press, Inc., Florida, USA, 1985, 25-35.
8. Drusano G.L. Friesen, A., Hoffken G., Vries-Hosper, H.G., Vander, W.D. The pharmacokinetics of trimethoprim-sulfamethoxazole. In: Bactrim Today, Zuckucwerdt, W. Germany, 1988, 12-22.
9. Rowland M., Tozer T.N. Estimation of the Elimination Half-life from Cumulative Urine Data In: Clinical Pharmacokinetics: Concepts and Applications, Lea and Febiger, Philadelphia, 1980, 291.
10. Brooks M.A., De Silva, J.A.F D'Arconte, L. Determination of trimethoprim and its N-Oxide metabolites in urine of man, dog and rat by differential pulse polarography. J. Pharm.Sci.. 62: 1973, 1395-1397.
11. James T.D., Joseph V.S. Biopharmaceutics Part 1: Factors influencing drug distribution in the Body. Am.J.Pharm. 8: 1965, 144-168.
12. Bach M.C., Gold O., Finland M. Absorption and Urinary Excretion of trimethoprim, sulfamethoxazole and trimethoprim-sulfamethoxazole: Results with single dose in normal young adults and preliminary observation during therapy with trimethoprim-sulphamethoxazole. The Inf. Dis., 12(S11): 1973, S584-S598.
13. Azuike, M.C. Essential Drugs List-Benefits and Problems. The Nig. J. Pharm., 1987,14-16.

Received: 2 April, 2002
Accepted: 21 September, 2004