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The Pharmacokinetic and Biopharmaceutical Effect of Ascorbic acid (Vitamin C) on Pefloxacin on Concurrent Administration in Human

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ABSTRACT

The aim of the study was to assess the pharmacokinetic and biopharmaceutical effect of ascorbic acid (Vitamin C) on pefloxacin on concurrent administration in man through urine excretion data and microbiological evaluation. A two way crossover study was performed in ten healthy male volunteers aged (Mean \pm SD) 45 ± 5.5 years and weight (Mean \pm SD) 75.4 ± 12.5 Kg recruited and given pefloxacin 400mg as single dose and urine samples collected and pooled up at time intervals. The drug was concurrently given with 500mg vitamin C after a washout period of 4 weeks and urine samples similarly collected. Urine samples collected were analyzed and pefloxacin concentrations were determined with UV spectrophotometer from a validated calibration curve. The pharmacokinetic parameters C_{max} , t_{max} , K_e and $t_{1/2}$ were determined and compared. Microbial evaluation of the interaction of the drugs was performed through MIC determination using urinary isolate *S. aureus* (U-11420). The k_e for pefloxacin alone was significantly lower than that for pefloxacin concurrently administered with vitamin C ($0.1hr^{-1}$ and $0.3hr^{-1}$) $P < 0.05$. The amount of pefloxacin excreted was significantly lower on single administration of pefloxacin compared to the co-administration with vitamin C, (44.13mg against 141.99mg) at $P < 0.05$. The MIC obtained against *S. aureus* was 0.025mg/ml for pefloxacin alone while the co-solution with vitamin C at below 2hr and 4hr impregnation period was 0.05mg/ml and 0.1mg/ml respectively. There was significant chemical, microbiological and biopharmaceutical interaction on co-administration of pefloxacin with vitamin C.

Keywords: Pefloxacin, vitamin C, Co-administration, Biopharmaceutical interaction.

INTRODUCTION

The co-administration of drugs believed to have benefits may in actual fact cause variable or unpredictable clinical response to the major drug (Kastrup, 2000). Pharmacokinetic interaction occurs when the absorption, distribution, metabolism or excretion of one drug is altered by another (Gibaldi, 1991). Clinically significant drug interactions are more likely to occur when a rapid peak plasma concentration of one of the drug is desired for therapeutic activity [Brodie and Fedy, 2000, Piscitelli, 2000 and Katzung, 1998].

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The same argument goes for drugs with short half life. Drug–drug interaction involves alteration of gastrointestinal pH values and formation of insoluble complexes, chelated compounds, sequestration of drug bound to bile acid, alteration of blood flow to the gastrointestinal mucosa, acceleration or decelerations of gastric emptying, change in vascularity or permeability of the mucosa or damage to the gut wall (Kurbacter, 1996).

Drug–drug interaction after absorptive phase involves interactions at the protein binding sites and other depots due to the relative affinity of the drugs to the affected tissues or macromolecules [Gibaldi, 1991]. Pefloxacin, a fluoroquinolone pyridine- β -carboxylic acid nucleus and broad spectrum synthetic bactericidal agent is often prescribed concurrently with ascorbic acid (Vitamin C) possibly due to its immunomodulatory, anti-oxidant and scavenging properties.

Ascorbic acid, the enolic form of 3-oxo-L-glucufuranolactone is arguably the most important water soluble biological antioxidant against reactive oxygen and nitrogen species (Lietman, 1995). Pefloxacin blocks bacterial DNA synthesis by inhibiting bacterial topoisomerase II (DNA – gyrase) and topoisomerase IV enzymes leading to bacterial cell rupture and death. The 3-carboxylate and 4-carbonyl groups are essential for antibacterial activity because they mediate the binding to the DNA gyrase complex while the C₆ fluorine enhances cell penetration [Lietman, 1995, Wetland, 1990 and Fernandez and Chu, 1990]. The oxido-reductive process involved in the mechanism of activity of pefloxacin may be affected by the effect of ascorbic acid on the redox potential of the system.

This work aims at determining the effect of concurrent administration of vitamin C with pefloxacin on the pharmacokinetics of pefloxacin and the possible drug-drug interaction affecting pefloxacin activity.

MATERIALS AND METHOD

Materials and Reagents

Hydrochloric acid, dimethylformamide, tetrabutylammonium hydroxide, methyl alcohol were products of BDH Chemicals, Germany. Mueller–Hinton agar was product of Quelab laboratories Inc. Canada. Pefloxacin tablets branded Peflacine[®] having label strength of 400mg (Table 1) and ascorbic acid (Vitamin C) label strength of 500mg were purchased from a retail pharmacy in Uyo, Akwa Ibom State, Nigeria. Pefloxacin and Vitamin C powdered samples were supplied by Orfen and Turex Pharmaceuticals, Lagos, Nigeria respectively.

Method

Tablet hardness

Ten tablets were randomly taken from the drug and placed on the edge of the tester and subjected to crushing strength of the tester.

Friability test

Twenty tablets were weighed and subjected to abrasion using a Veego tablet friability tester at 25 rev/min.

Uniformity of weight

Ten tablets were taken from the Peflacine brand and weighed individually. The mean and percentage deviation was determined.

Disintegration time

Six tablets were placed in the tubes. The end of the tubes were lowered and raised in a bath of SIF and SGF maintained at 37 \pm 1°C. The time when no drug particle was found on the screen was noted and recorded. This was repeated for the FMSIF and FMSG Tablet Disintegration

Tablet disintegration was determined at 37°C using a Veego model VTDH3 disintegration testing apparatus (Rutartek, India)

Chemical content determination

The chemical content determinations of the pefloxacin pure drug sample was performed by weighing 0.2g of pefloxacin powdered drug product equivalent to 0.125g of pure drug and dissolved in 20ml of dimethylformamide and titrated with 0.1N tetrabutylammonium hydroxide. The end point was determined with 0.2% solution of thymolphthalein in methyl alcohol (Osadebe and Akabogu, 2004). The pure drug was similarly assayed. A calibration curve was derived from the plot of absorbance against concentration at 277 nm from the solution obtained by weighing 0.125g of the pure drug and dissolved in 20ml of methylalcohol and made up to 100ml with distilled water and further dilutions to obtain percentage concentrations in the range of 0.125% to 0.00125%. The drug content of the 5ml samples obtained at time intervals from the dissolution rate were determined by extrapolation from the calibration curve. The drug content of pefloxacin infusion was similarly determined. The chemical content of the vitamin C injection was obtained by dissolving 0.1g of vitamin C powdered drug sample in a mixture of 20 ml of 1M sulphuric acid and 20 ml of water and titrated with 0.1M ceric ammonium sulphate solution (Guideline, 1996). The employed vitamin C injection was similarly determined by pipetting 5ml of the solution and adding 10ml of 1M sulphuric acid and 10ml of water before titrating with 0.1M ceric ammonium sulphate solution.

Volunteers and clinical protocols

The study protocol and the informed consent forms were approved by the Ethical Committee of the University of Uyo Health Services, Uyo, Nigeria. The whole study which meets the requirements of the declarations of Helsinki was conducted in accordance with the Current Good Clinical Practice (GCP), International Conference Harmonization (ICH) as well as Good Laboratory Practice (GLP) Guidelines. (WHO, 2009 and Munro, 1996).

Ten volunteers were examined and passed through a detailed history taking, physical examination, biochemical investigations (liver and renal function) haematological examination (%Hb, PCV and ESR). There was none of the subjects with contraindication to pefloxacin. None of the subjects was a

smoker and had no medication in the space of two weeks prior the study.

Drug Administration

400mg of pefloxacin tablet was administered to each of the ten volunteers and the urine voided were collected and pooled together for time intervals of 30 min, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours and 24 hours. The total volume for each time interval was noted and 5ml representative sample was retained for analysis using the UV spectrophotometer. A washout period of 4 weeks was observed before the same protocols were observed with 400mg of pefloxacin and 500mg of ascorbic acid concurrently administered to the volunteers.

Microbial Evaluation

A microbiological assay of the antibacterial agent was performed to determine the comparative MIC using urine isolate *S. aureus* (11420) against pefloxacin and co-solution of pefloxacin with ascorbic acid.

Serial dilutions of pefloxacin was made from a stock prepared from pefloxacin infusion and all dilutions made in phosphate buffer (pH 7.4) to give concentrations of 0.1, 0.05, 0.025, 0.0125 and 0.0075 µg/ml. 1ml of each of the concentrations of pefloxacin prepared was diluted with 1ml of sterile water and used to impregnate the sensitivity discs. Vitamin C injection was diluted to give 0.01mg/ml and 1ml solution was added to 1 ml of the prepared concentrations of pefloxacin. Another set of serially diluted pefloxacin both to maintain the concentration of pefloxacin as earlier indicated. Discs were similarly impregnated with the resulting solutions of the control and the co-solution of vitamin C and pefloxacin.

The *S. aureus* strain used was subjected to preliminary sensitivity testing to pefloxacin using discs containing 2 µg/ml of the antibiotic. The microbiological study was done in a single blind fashion in which the drug pattern were coded A', 'B and 'C' so that the interpreter for the growth on the plates did not know the drug composition. The plates were incubated at 37°C for 24 hours (NCCLS,1990, Wood, 1995 and Rubson 1992).

Statistical Analysis

Statistically significant difference in the amount of pefloxacin absorbed when administered alone and on concurrent use with ascorbic acid as analyzed using one sample hypothesis and two tailed with $\alpha=0.05$ employing Statistical Package for Social Scientists (SPSS) version 17.

RESULTS

The details of the drugs used in the study are stated out in Table 1. The outcomes of the physicochemical tests on pefloxacin are set out in Table 3 and 4. The calibration curves constructed from the pefloxacin and vitamin C parenteral dosage forms for drug concentration and corresponding absorbance were linear from 0.01 to 100mg. The details of the slope, intercept and correlation coefficient are expressed in Table 2. The mean \pm S.D of pefloxacin

excreted in urine for both drug administration are expressed in Fig 1. The C_{max} of pefloxacin calculated for pefloxacin administered alone was significantly higher than that for concurrent administration with Vitamin C ($P<0.05$). The average amount of pefloxacin excreted when administered alone was 44.13 ± 7.92 mg in 24 hours while it was 141.99 ± 17.26 mg on concurrent administration with Vitamin C. The k_{el} was 0.1hr^{-1} and 0.3hr^{-1} for the drug administration's respectively. The lowest concentration of pefloxacin that inhibited the growth of the test organisms are expressed in Table 5.

Table 1: Details of the drugs used in the study.

	Pefloxacin	Vitamin C
Manufacturer	May and Barker	Juhel, Nigeria
Country of production.	France	Nigeria
Batch No	119	0665
Registration No.	04 – 1015	04 – 1070
Manufacturing Date	12 – 2003	01 – 2005
Expiry Date	12 – 2006	01 – 2006

Table 2: The calibration curve parameters of the drugs.

Calibration curve parameters	Pefloxacin	Vitamin C
Slope	1.59	2.45
Intercept	0.003	0.007
Correlation coefficient	0.986	0.994

Table 3: Chemical content determination of the employed samples

	Percentage Content of Drug in Sample			
	Sample 1	Sample 2	Sample 3	Mean \pm SD
Pefloxacin powdered drug product	89.22	96.54	103.45	96.40 \pm 7.12
Pefloxacin pure drug	94.63	97.54	99.63	97.27 \pm 2.50
Pefloxacin infusion	90.12	91.06	94.24	91.80 \pm 2.16
Vitamin C injection	85.96	90.31	84.20	90.31 \pm 3.10

Table 4: The physicochemical properties of pefloxacin brand employed.

Hardness test (Kg/cm ²)	Friability test (%)	Weight uniformity (g)	Disintegration test in SGF (min)
1.3 \pm 0.2	0.034	565.40 \pm 0.05	6.5 \pm 0.3

Table 5: The mean MIC of drug against *S. aureus* (U-11420) against time from 1hr to 4hr.

Drugs	Minimum Inhibitory Concentration (MIC) mg/ml			
	1hr	2hr	3hr	4hr
Pefloxacin	0.025	0.025	0.025	0.025
Pefloxacin + Vit. C	0.05	0.05	0.1	0.1

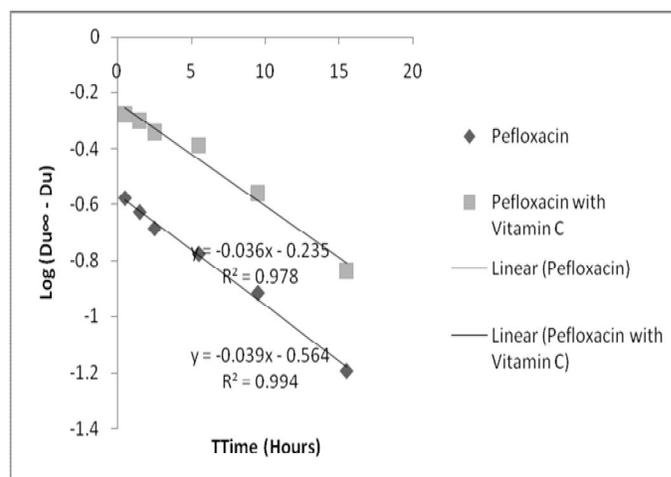


Fig. 1: The semi-logarithmic plot of $D_u^\infty - D_u$ versus time for urine samples collected.

DISCUSSION

The physicochemical tests performed on the tablets of peflaxine had results consistent with the official specifications which is an indication of the appropriateness of the drug sample used for the pharmacokinetic and biopharmaceutical assessment of the concomitantly administered pefloxacin and vitamin C [Sadec and Belan, 1990]. Error may be introduced to the analysis from the angle of the physicochemical profile of the employed drug giving misleading results. Ten male volunteers were recruited into the study and thorough medical examinations were conducted to ascertain their health status which may also affect the disposition of drugs. The urine voided was pooled over the 24 hours period of sample collection and no urine volume was lost. Loss of urine samples leads to drug loss and this is significant in pharmacokinetic determination using urinary excretion data. The analysis performed was based on the quantification of the unchanged and metabolized fraction of pefloxacin as the UV spectrophotometer does not selectively detect the metabolized and the unchanged fraction of the drug at the wavelength of detection. The mean amount of pefloxacin excreted when given alone was significantly lower than that when co-administered with vitamin C (44.13 mg and 141.99 mg respectively at $P < 0.05$). This connotes a facilitated elimination of pefloxacin in the presence of vitamin C [18-20]. Absorption and excretion of drugs involves the passage of molecules of drugs across biological membranes and the presence of a co-administered substance such as vitamin C may accelerate or hinder the free passage of the former through membranes. There was significant difference in K_{el} pefloxacin compared with pefloxacin and vitamin C co-administration (0.1hr-1 and 0.3 hr-1) and the half lives being 6.93 hr and 2.3 hrs respectively. Co-administration of vitamin C with pefloxacin therefore facilitates the absorption of pefloxacin and in the same vein facilitates the rapid excretion of the same through the renal route. Microbiological assay evaluating the effect of vitamin c on pefloxacin in the presence of a urinary isolate of *S. aureus* was designed to assess the lethal effect of the later in the presence of the former. It was observed that the presence of vitamin C led to an increase in MIC of pefloxacin and this progressively increased with time. A chemical reaction was thought to occur between the chemical species in solution. The impregnated disc for the microbiological evaluation of pefloxacin was therefore based on the amount of pefloxacin effectively present for impregnation (Bressole *et al*, 1994).

CONCLUSION

Pefloxacin is required to be available in sufficient amount and reasonable time to give a good bacterial clearance. The facilitated absorption and excretion of pefloxacin in the presence of

vitamin C therefore may affect the drug disposition and calls for careful consideration on concurrent prescribing.

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