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Full Length Research Paper

Effect of artesunate on the dissolution profile and antimicrobial activity of ciprofloxacin hydrochloride

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This study evaluated the *in vitro* physico-chemical implication of co-presentation of ciprofloxacin with artesunate. Thin layer chromatography (TLC) and ultraviolet (UV) analysis was used to monitor the co-presentation of the two drugs. The *in vitro* dissolution profile of ciprofloxacin hydrochloride tablet in the presence of artesunate tablet was evaluated in buffer with pH 1.2 and water at 37± 0.5°C as well as the effect artesunate on the antibacterial activity of ciprofloxacin. Physico-chemical interaction was not observed between ciprofloxacin and artesunate. However, a significant reduction in the dissolution rate of ciprofloxacin was observed in buffer pH 1.2 solution when compared with water as the medium ($p < 0.01$). Furthermore, the rate of dissolution of ciprofloxacin was significantly reduced in the presence of 200 mg artesunate in both media ($p = 0.0164$). Similarly, the extent of drug release at 30 min (C_{30}) was generally reduced in the presence of 200 mg artesunate in both water and buffer pH 1.2 solution ($p < 0.05$). The reduction in antimicrobial activity of ciprofloxacin on clinical isolates of *Staphylococcus aureus* and *Escherichia coli* was observed to be statistically insignificant in the presence of artesunate ($p > 0.05$). The results obtained in this study suggest that care should be taken during the co-administration of ciprofloxacin with the starting dose of artesunate.

Key words: Ciprofloxacin, artesunate, physico-chemical interaction, dissolution rate, *Staphylococcus aureus* and *Escherichia coli*.

INTRODUCTION

Ciprofloxacin [1 cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid], is one of the quinolone antibiotics which is a class of highly potent and orally active broad spectrum antibacterial compounds developed from the original 1,8-napthyridine urinary antibacterial agent, nalidixic acid (Smith et al., 1990). Ciprofloxacin is indicated for both Gram-negative and Gram- positive bacterial infections. It has been used in the treatment of a wide range of infections including biliary-tract infections, bone and joint infections, acute bacterial infections, gastro-enteritis, gonorrhea, immuno-compromised patients (neutropenia), skin disorders, typhoid and paratyphoid fever and urinary-tract infection (UTI) (Martindale, 2009). Ciprofloxacin have been reported to inhibit DNA gyrase activity, thereby causing significant antiparasitic effect against various strains of *Plasmodium falciparum* malaria (Mahmoud et al, 2003; Yeo and Rieckmann 1994). Malaria, a parasitic protozoal disease, is one of the most serious complex and refractory health problems currently facing humanity. The co-presentation of microbial infections and malaria is not uncommon in tropical developing countries, thus, the co-administration of antimicrobial drugs with antimalaria is a common practice. The co-administration of two or more drugs is usually accompanied by a variety of therapeutic implications ranging from opposition, alteration, synergism, potentiating as well as physical and chemical antagonism (Olaniyi, 2000). 3R,5aS,6R,8aS,9R,-10S,-12R,12aR)-decahydro-3,6,9-trimethyl-3,12-epoxy-12H pyrano(4,3-j)-1,2-benzodioxepin-10-ol hydrogen Artesunate, chemically known as (3R,5aS,6R,8aS,9R,10S,12R,12aR)-decahydro-3,6,9 trimethyl-3,12-epoxy-12H-pyrano[4,3-j]-

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1,2 enzodioxepin-10-ol hydrogen succinate, is a derivative of artemisinin which have been found useful in chloroquine-resistant malaria (O'Neill et al., 2010). Artesunate is about the most widely used of the artemisinin derivatives and is a key member of all the artemisinin combination therapies (ACT) compositions recommended by World Health Organization (WHO) for the management of malaria presently (WHO, 2006). The co-administration of ciprofloxacin with a variety of drugs has been reportedly accompanied with varied pharmacological effects. Probenecid was reported to increase the plasma concentration of ciprofloxacin as a result of a reduction of its excretion (Lim et al., 1995). Cyclosporine increases risks of nephrotoxicity when used concomitantly with ciprofloxacin (Van et al., 1990), while non-steroidal anti-inflammatory drugs (NSAIDs) and theophylline possibly increases risk of convulsion with ciprofloxacin (Polk, 1989). Antacids, calcium salts, sucralfate, zinc and oral iron reduce the absorption of ciprofloxacin; this may result in decreased plasma concentration (Taxeira et al., 1995; Joint Formulary Committee, 2003).

The possibility of decreased solubility resulting in decreased absorption as a result of physical interaction due to adsorption of drug particles has been reported (Galia et al., 1998). Similarly, chemical interactions in form of oxidation, hydrolysis, etc. of two or more drugs may result in significant effect on the therapeutic activity of the drug compounds (DiPalma, 1976). The co-administration of ciprofloxacin with antimalaria agents such as chloroquine and artesunate during the management of bacterial infection co-presented with malaria as a result of its broad spectrum of activity formed the basis for this study. In this study, the effects of artesunate on the dissolution profile and antimicrobial activity of ciprofloxacin against two strains of *Staphylococcus aureus* and *Escherichia coli* were determined.

MATERIALS AND METHODS

Materials and equipment

Ciprofloxacin hydrochloride powder was donated by Gemini Pharmaceuticals, Lagos, Nigeria, while artesunate powder was donated by Bond Chemical Industries Ltd, Awe, Oyo State, Nigeria. Artesunate tablet (50 mg) manufactured by Makophar Chemical Pharmaceutical Company Vietnam and ciprofloxacin HCl tablets (500 mg) manufactured by V.S. International PVT.LTD were obtained from retail pharmacy outlets in Ibadan. All the reagents used were of analytical grade.

UV Spectrophotometer (UNICAM) was use for drug content quantization, while Langford ultrasonics sonomatic® water bath and dissolution test apparatus USP standard were used for the interaction studies. The microbiological assay was carried out using Fisher Isotemp $^{(R)}$ 200 series incubator and Equitron autoclave carried out by determining the melting point, UV spectra between 200 to 400 nm. The chemical content were determined using the official method for artesunate (I.P. 2003), while an earlier reported method with very good interday and intraday precision was used for ciprofloxacin HCl samples (Adegbolagun et al., 2007).

Physico-chemical interaction evaluation

Ultraviolet spectroscopy

Stock solutions of ciprofloxacin HCl (5 mg/ml) (CIP) and artesunate (1 mg/ml) (ART) were prepared in buffer pH 1.2. Aliquot concentrations; 1000 µg/ml of CIP, 100 and 200 µg/ml of artesunate (ART) were prepared from the stock solutions. Solutions of the two drugs were combined at the following ratios to give a final concentration of; ART: CIP (50: 500 µg/ml and 200: 500 µg/ml), also individual solutions at ART (50 and 200 µg/ml) and CIP (500 µg/ml) were transferred into six labeled covered test-tubes per reaction mixture. The test tubes were placed in thermostated water bath at 37± 0.5°C. UV spectra (200 to 400 nm) of each set of the reaction mixture were run at 0, 15, 30, 45, 60 and 90 min using the buffer pH 1.2 as blank. The determination was carried out in duplicate. The procedure was repeated in distilled water.

In vitro dissolution profile evaluation

UV absorbance of solutions of CIP (0.1 - 5 × 10^{-4}%w/v) at 276 nm was used to generate a calibration curve of CIP in buffer pH 1.2 and distilled water. *In vitro* dissolution profile of CIP was determined according to B.P. 2010 method (British Pharmacopoeia, 2010). The determination was carried out in water at 37± 0.5°C using the one tablet (500 mg) and in the presence of artesunate tablets at 50 and 200mg. Aliquots of 10 ml were withdrawn at 10 min interval for 60 min and assayed. The dissolution fluid volume was maintained by adding 10 ml of the dissolution medium which had previously been maintained at 37± 0.5°C after each withdrawal. The withdrawn samples were assayed using UV spectrophotometer at 276 nm using the dissolution medium as blank. The procedure was repeated with four determinations, using buffer pH 1.2 at 37± 0.5°C as the dissolution medium.

Antimicrobial interaction evaluation

The effect of the *in vitro* co-presentation of artesunate and ciprofloxacin was evaluated on two strains each of *S. aureus* and *E. coli*. The bacterial strains include one clinical isolates of *E. coli* and *S. aureus* and their reference standards; American Type Culture Collection (*E. coli* ATCC 25922 and *S. aureus* ATCC 14563) obtained from the University College Hospital, Ibadan, Nigeria were used for the studies. All the bacterial cultures were grown in sterile nutrient broth at 37± 0.5°C for 24 h and maintained in nutrient agar at 4°C until use.

Sensitivity test

Overnight cultures (24 h) of *E. coli* and *S. aureus* (0.2 ml) were prepared by serial dilutions. Aliquots solutions (0.2 ml) of CIP (1.5625 × 10^{-3} – 4.0 µg/ml) was transferred into Mueller-Hinton broth (9 ml) followed by 0.2 ml of overnight culture of the bacterium and 0.6 ml of nutrient broth. The tubes were mixed and incubated at37°C for 24 h. The negative control contains the sterile broth, while the positive control contains the sterile nutrient broth, 0.8 ml sterile distilled water and 0.2 ml of the bacterium. The minimum inhibitory concentration (MIC) was defined as lowest concentration of the drug at which no turbidity was observed. The determination was done in triplicates. The procedure was also repeated with similar concentrations of artesunate.

Antimicrobial assays

Aliquots solutions of ciprofloxacin hydrochloride at 0.5, 1.0, 2.0 and

Table 1. Experimental designs for the antimicrobial drug - drug interaction studies between ciprofloxacin hydrochloride and artesunate.

CIP (µg/ml)	ART (µg/ml)			
	0.0	**0.1**	**0.2**	**0.3**
0.0	0.0/0.0	0.0/0.1	0.0/0.2	0.0/0.3
0.5	0.5/0.0	0.5/0.1	0.5/0.2	0.5/0.3
1.0	1.0/0.0	1.0/0.1	1.0/0.2	1.0/0.3
2.0	2.0/0.0	2.0/0.1	2.0/0.2	2.0/0.3
4.0	4.0/0.0	4.0/0.1	4.0/0.2	4.0/0.3

CIP, Ciprofloxacin hydrochloride; ART, artesunate.

4.0 µg/ml was prepared from the stock solution in buffer pH 1.2, while those of artesunate was similarly prepared at 0.1, 0.2, 0.3 µg/ml from its stock solution in buffer. Diluted overnight cultures of the different micro organism (0.2ml) was individually introduced into 20 ml of warm molten agar, which was carefully mixed and poured into sterile Petri plates and allowed to set for about 45 to 60 min. Wells of 8 mm were aseptically cut in the set agar in Petri plates inoculated with the microbial suspension.

The pure drugs (CIP and ART) and their mixtures at the different combination ratios (Table 1) were prepared in test tubes which were covered and placed in thermostated water bath set at 37 ±0.5°C. Samples (0.2 ml) were carefully withdrawn from the reaction mixtures and transferred into the labeled wells of the seeded agar plates at 0, 15, 30, 45 and 60 min. The agar plates were kept for 1 to 2 h to allow diffusion before incubation at 37 ±0.5°C for 24 h. The diameters of zones of inhibition were measured in mm. The procedure was done in triplicates and also repeated for the drug solutions in distilled water.

Statistical analysis

The results obtained in the research work were expressed as mean ± standard deviation. The results were analysed using one way ANOVA and Student t-test (Instat 3, Statistical Package) with $p< 0.05$ for level of significance.

RESULTS AND DISCUSSION

The identification and chemical content determination of the drug compounds authenticated the quality of the two drug compounds. CIP melted at 240 – 241°C with a UV λ_{max} at 276 nm, while the melting point of the pure ART was 131 - 132°C and did not show any UV absorbance between 210 to 400 nm. Their chemical contents obtained were also within the official specification for the two drugs [ciprofloxacin HCl (100.8 and 98.9%w/w); artesunate (100.5 and 96.2%w/w) for pure drug and tablet dosage form, respectively] (I.P. 2003, B.P. 2010). The physico-chemical interaction evaluation monitored by using the UV spectrophotometer did not reveal any effect on the UV spectrum of CIP as there was no change in the λ_{max} and absorbance reading at 276 nm in the presence of ART. This may indicate an absence of observable physicochemical interaction between CIP and ART.

Dissolution of drugs is a very important step in the absorption of drugs from dosage forms. It is one of the fundamental parameters controlling the rate and extent of drug absorption (Gordon et al., 1995). Dissolution profiles of some drugs have been reportedly affected by the dissolution medium (Galia et al., 1998; Lobenberg et al., 2000). Buffer pH 1.2 was used in this study to provide a similar medium to the normal pH of the stomach which is the site of drug dissolution prior to absorption. On the other hand, water was selected as the second medium because it is the specified medium for the evaluation of the dissolution profile of CIP (B.P. 2010). The experimental design for the dissolution profile evaluation used CIP tablet (500 mg), while ART at 200 mg is the starting dose for the management of malaria while 50 mg was used to observe the effect at the lower concentration of ART.

From the results obtained, the official monographs specified an *in vitro* release of 80%w/v for CIP at 30 min; that is, the time to release/dissolve 80%w/v of the drug (T_{80}) was 30 min. The dissolution profile of CIP in buffer pH 1.2 and water showed a delayed rate of release from the tablet dosage form in the presence of ART (Figure 1; Table 2). The rate of release of drugs from dosage forms was obtained from the time to reach 80%w/v concentration (T_{80}). A significant increase in T_{80} of CIP alone in buffer pH 1.2 compared with water was observed in this study with a T_{80} of 12.0 ± 4.5 and 35.3 ± 2.2 min in water and buffer, res-pectively ($p = 0.0013$). This indicates a significant reduction in the rate of release of CIP in buffer solution. However, no significant difference was observed in the % CIP released at 30 min (C_{30}) in the two media in the absence of ART ($p > 0.05$).

Furthermore, a significant increase in T_{80} of CIP was observed in the presence of 200 mg ART in both water and buffer solution ($p < 0.05$). This indicates a significant reduction in the rate of release/ dissolution from the tablet dosage form in both media. The specified *in vitro* dissolution profile of CIP tablet dosage form should be 80%w/v at 30 min (T_{80} of 30 min and C_{30} of 80%w/v) (B.P. 2010). A highly significant increase in T_{80} of CIP was observed in the presence of 200 mg ART when compared with 50 mg in both media ($p < 0.05$). Moreover, the CIP release in the presence of 200 mg ART at 30 min was lower than specified 80%w/v in both media (Table 2), while the time to achieve 80%w/v dissolution was also

Table 2. Dissolution profiles (T_{80} and C_{30}) of ciprofloxacin hydrochloride tablet in the presence of artesunate tablets at 50 and 200 mg (mean ± S.D.).

Sample	T_{80} (% ± S.D.)		C_{30} (min ± S.D.)	
	Buffer pH 1.2	Water	Buffer pH 1.2	Water
CIP alone	35.3 ± 2.2	12.0 ± 4.5	73.3 ± 1.3	89.8 ± 14.8
CIP + 50 mg ART	25.6 ± 11.9	13.7 ± 8.4	83.9 ± 10.9	97.9 ± 7.5
CIP + 200 mg ART	53.5 ± 7.6	46.5 ± 0.9	21.7 ± 6.9	65.9 ± 6.1
p value (ANOVA)	0.0164	0.0029	0.0001	0.0565

CIP, Ciprofloxacin hydrochloride; ART, artesunate.

Table 3. Zones of inhibition (mm) of ciprofloxacin hydrochloride (CIP) (0 – 4ug/ml) in the presence of artesunate (ART) (0.1 – 0.3ug/ml) at 0 and 60minutes against two strains of *S. aureus* and *E. coli*.

Organism	ART concentration (ug/ml)	Zone of inhibition at different concentrations of CIP (ug/ml) at 0 and 60 min (mm)							
		0.5		1.0		2.0		4.0	
		0	60	0	60	0	60	0	60
SAT	0.1	15.7 ± 3.21	17.7 ± 1.15	22.3 ± 3.51	22.0 ± 0.1	23.3 ± 2.1	23.5 ± 2.0	24.7 ± 2.1	23.5 ± 0.7
	0.2	20.0 ± 2.82	16.3 ± 7.23	18.7 ± 3.2	20.3 ± 3.5	22 ± 1.0	22.5 ± 3.5	26.3 ± 1.5	24.0 ± 2.7
	0.3	18.3 ± 2.08	22.3 ± 2.31	18.3 ± 7.0	26.7 ± 0.6	21.7 ± 1.2	21.0 ± 1.0	24.7 ± 0.6	24.7 ± 2.9
SAC	0.1	17.3 ± 3.0	16.3 ± 3.1	22.7 ± 4.7	20.3 ± 1.5	22.7 ± 4.0	17.7 ± 5.7	28.0 ± 2.0	23.7 ± 3.8
	0.2	20.7 ± 0.6	11.3 ± 1.5	21.3 ± 4.2	12.0 ± 5.0	20.3 ± 0.6	21.0 ± 1.0	28.0 ± 3.6	22.7 ± 0.6
	0.3	19.7 ± 4.5	16.7 ± 1.5	24.0 ± 1.7	18.3 ± 2.5	22.7 ±2.5	21.0 ± 1.0	27.7 ± 2.1	23.3 ± 1.5
ECT	0.1	24.3 ± 1.5	25.7 ± 5.5	28.3 ± 1.5	22.3 ± 1.2	30.7 ± 0.6	29.7 ± 1.5	31.0 ± 3.5	32.0 ± 1.0
	0.2	24.7 ±1.5	20.7 ± 2.1	30.7 ± 4.0	28.7 ± 4.9	30.7 ± 1.2	26.3 ± 7.7	26.0 ± 4.4	30.7 ± 1.5
	0.3	24.3 ± 3.2	22.0 ± 5.1	32.3 ± 4.1	27.3 ± 4.6	31.7 ± 1.5	26.7 ± 1.5	31.0 ± 2.7	33.0 ± 1.0
ECC	0.1	26.0 ± 4.0	27.3 ± 2.3	33.7 ± 2.9	30.0 ± 2.5	32.0 ± 1.0	27.7 ± 0.6	34.7 ± 0.6	33.3 ± 0.6
	0.2	33.3 ± 3.2	25.0 ± 3.0	30.7 ± 1.2	28.7 ± 2.5	33.7 ± 2.9	28.7 ± 1.2	36.7 ± 0.6	28.0 ± 1.7
	0.3	32.7 ± 2.5	22.0 ± 1.0	31.7 ± 2.5	31.0 ± 1.7	30.7 ± 1.2	26.7 ± 2.1	37.0 ± 1.0	31.3 ± 3.1

higher than the 30 min in the presence 200 mg ART. The increased T_{80} and decreased C_{30} with ART at 200mg imply an increase in the time to achieve appreciable biological concentration as a result of delayed rate of *in vivo* drug absorption. This may cause a decrease in the rate of achieving desired therapeutic effect, which may result in a compromise of the therapeutic outcome. On the other hand, the significant difference in the dissolution profile of CIP alone in buffer pH 1.2 and water is an indication that the use of water to define the dissolution profile may not be a good indicator of the dissolution profile of CIP in biological medium.

In addition, the microbial sensitivity test showed that the micro organisms used in this study were sensitive to the CIP at 0.25 to 4.0 µg/ml, while ART expectedly did not show any antimicrobial activity even at the highest concentration of 0.4 µg/ml. The effect of ART on *in vitro* antimicrobial effect of CIP on *E. coli* and *S. aureus* revealed a decrease in the antimicrobial activity on the clinical isolates which were however found to be statistically insignificant ($p > 0.05$) (Table 3).

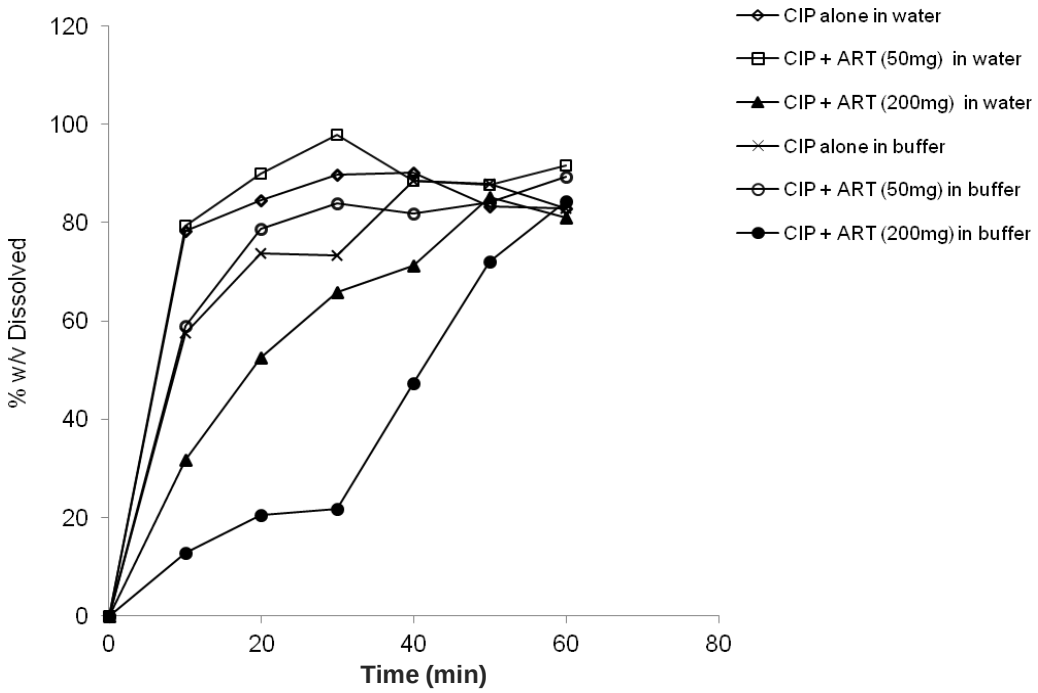

Figure 1. Dissolution profile of ciprofloxacin hydrochloride tablet (500 mg) in the presence of artesunate tablet at 50 and 200 mg in water and buffer pH 1.2 at 37± 0.5°C (CIP: ciprofloxacin hydrochloride; ART: artesunate).

Nevertheless, no difference in antimicrobial activity was observed with the reference micro organisms (SAT and ECT) across the different concentrations used in this study.

Conclusion

Although no significant difference was obtained with the physico-chemical interaction study, the alteration in the dissolution profile of CIP in the presence of 200 mg ART calls for caution during the administration of CIP with starting dose of ART. This is further important in view of the obtained result of non-statistically significant reduction in antimicrobial activity obtained with the clinical isolates. It is hereby suggested that care should be taken during the co-administration of CIP with the starting dose of ART. An adequate time interval should be observed in order to avoid compromising the therapeutic outcome.

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