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In Vitro Activity of Chloroquine Phosphate on the Antibacterial Potency of Ciprofloxacin Hydrochloride on the Clinical Isolates of Some Gram-Negative Microorganisms

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ABSTRACT

The in vitro activity of chloroquine on the antibacterial effect of ciprofloxacin on some Gram-negative organisms was investigated using the in vitro standardized method of the National Committee for Clinical Laboratory Standard (NCCLS). The study showed that three of the four strains of *Pseudomonas aeruginosa* (PA_1, PA_2, and PA4) and the strain of *Klebsiella pneumoniae* (KP) isolates were sensitive to ciprofloxacin hydrochloride alone as well as in combination with chloroquine phosphate. However a strain of *Pseudomonas aeruginosa* (PA_3) was sensitive to ciprofloxacin hydrochloride alone, but the sensitivity to ciprofloxacin hydrochloride was lost in the presence of chloroquine even at the lowest concentration of 1µg/ml. The two strains of *Escherichia coli* used were not sensitive to ciprofloxacin alone and in combination with chloroquine. There were no significant pharmacodynamic interaction between chloroquine and ciprofloxacin on the PA_1, PA_4 and KP strains used in vitro ($p>0.05$). On the other hand, a significant enhancement of in vitro antibacterial activities of ciprofloxacin by chloroquine on the strain PA_2 at the various concentrations was observed ($p<0.05$). The inhibition, enhancement or lack of effect of chloroquine phosphate on the ciprofloxacin hydrochloride observed in this study depends on the type and strain of microorganism involved.

Key Words: Ciprofloxacin, Chloroquine, Antibacterial effects, Gram-negative organisms.

INTRODUCTION

Drug-drug interaction occurs whenever the expected therapeutic response of a drug is modified by the concurrent or prior use of some other drug or combination of drugs (Wilson et al 1971). This interaction can occur by a direct or indirect mechanism. The modified response may be an increase in drug activity such as chloroquine enhancing the antibacterial activity of streptomycin (Crowle and May 1990); decrease in drug activity as in chloroquine competitively inhibiting the accumulation and activity of iron resulting in death of yeast cells (Emerson et al 2002) as well as absence of clinically relevant pharmacokinetic interaction during co-administration of chloroquine and azithromycin (Cook et al 2006). The increased activity of streptomycin (an antibacterial agent) in the presence of chloroquine suggests that chloroquine may have antibacterial enhancing potency on streptomycin (Crowle and May 1990).

Ciprofloxacin is a preferred oral agent for the treatment of *Pseudomonas aeruginosa* in urinary tract infections (Lebel 1988; Talan et al 2000) and cystic fibrosis complicated with Pseudomonas, Klebsiella and Escherichia infections (Zabranjecki et al 1996; Alghasha and Nahata 2000). It belongs to a class of antimicrobial frequently used in adult population to which sensitivity rates have remained consistently high.

Although a wide range of drugs are currently in use in the management of malarial infection, chloroquine is one of the most widely consumed anti-malarial drugs (Foster 1994). The prevalence of urinary tract infection (UTI) or upper respiratory tract infection (URTI) during malaria management is not uncommon in a malaria endemic area like sub-Saharan Africa.

An on going study on the rate of co-administration of ciprofloxacin and chloroquine during the management of malaria infection complicated by urinary tract or upper respiratory tract infections has revealed a significant rate of prescription of the two drugs together when compared to other antimalarial/antibacterial drug combinations among the population under investigation. Furthermore, a significant enhancement of the in vitro antiparasitic effect of chloroquine by ciprofloxacin has been reported (Kazzim et al 2006). However, the nature and mode of such interactions as well as the possible clinical implication(s) of such interactions during the treatment of malaria complicated by urinary tract or upper respiratory tract infections has not been reported.

This study was designed to investigate the effect of chloroquine on the antibacterial activity of ciprofloxacin in vitro, with the aim of identifying the nature of the effect of chloroquine, if any, on the antibacterial potency of ciprofloxacin.

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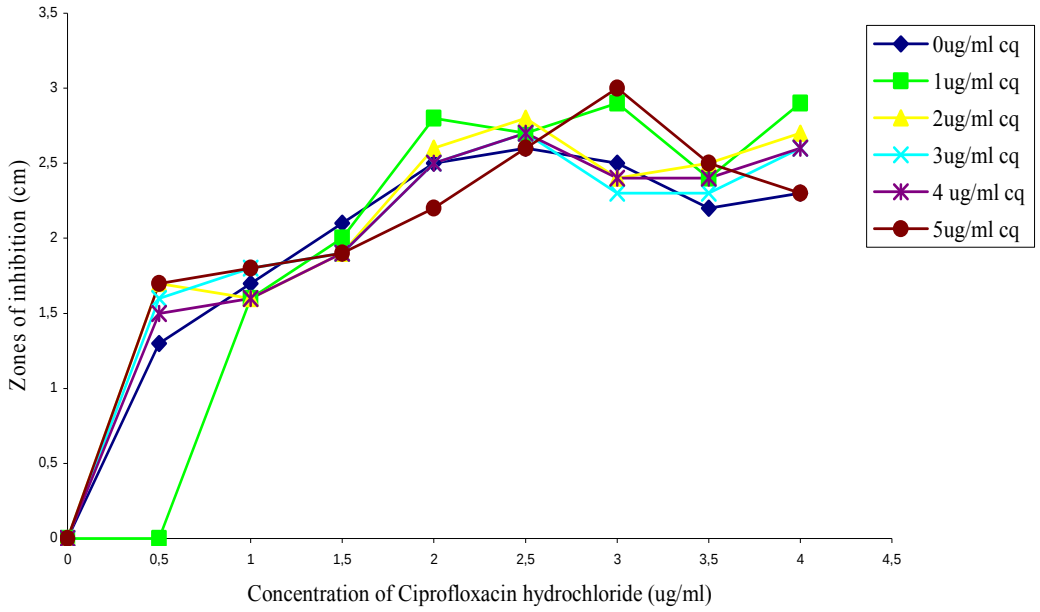

Figure 1. Zones of inhibitions of the pure ciprofloxacin and in combination with chloroquine against P. aeruginosa (PA_1)

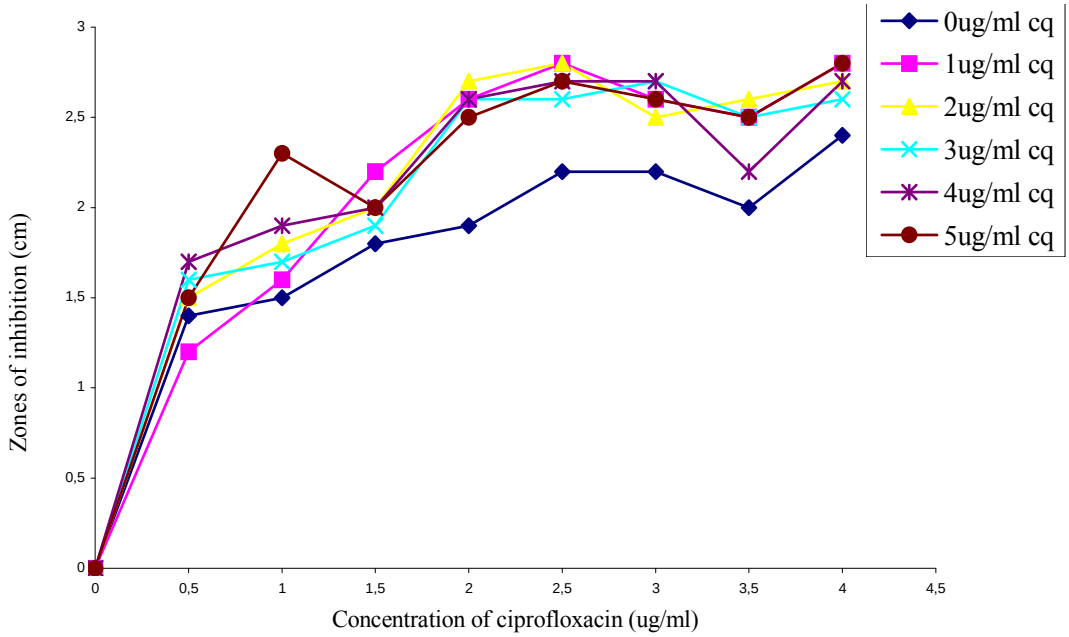

Figure 2. Zones of inhibitions of the pure ciprofloxacin and in combination with chloroquine against P. aeruginosa (PA_2)

MATERIALS AND METHODS

Quality evaluation of the pure drug compound

The pure ciprofloxacin hydrochloride and chloroquine phosphate powder were analyzed using the official methods of analysis specified for each drug compound, i.e. B.P. 1998 and U.S.P. 2000 respectively.

Preparation of stock solutions

0.1g of ciprofloxacin dissolved in 1ml ethanol was diluted to 10ml with sterile distilled water to form the initial stock solution for ciprofloxacin. From this stock solution, (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0)µg/ml concentrations of ciprofloxacin were prepared when needed. Similarly, 0.01%w/v solution of chloroquine phosphate in sterile distilled water was prepared as the initial stock solution for chloroquine. From this, (1.0,

2.0, 3.0, 4.0 and 5.0)µg/ml of chloroquine phosphate solutions were prepared when needed. The stock solutions were stored at -20^{o}C.

Identification and characterization of the microorganism

Bacteria used in this study were recently isolated clinical strains obtained from Medical Microbiology department of the University College Hospital, Ibadan, Nigeria. These bacteria were further characterized and identified by various microbiological and biochemical tests as well as with the aid of Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons 1974) and Singleton's Bacteria, Biotechnology and Medicine (Singleton 1998). Bacteriological tests such as Gram staining and morphological characterization as well as biochemical tests such as indole, methyl-Red-Voges-Proskauer, citrate, urease, H_2S production, motility and oxidase tests were also carried out.

Three well-isolated colonies of the same morphological type of each species were selected from nutrient agar plate culture. The surface of each colony was touched with a loop, and the growth is transferred into a tube containing 10ml of Mueller Hinton broth medium. The broth culture was incubated at 35°C for 6 hours until it achieves or exceeds the turbidity of the 0.5 McFarland standards. The turbidity of each actively growing broth culture was adjusted with sterile broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard using spectrophotometer OD625 of 0.08 - 0.1 (1 cm light path) to give a suspension containing approximately 1 to 2 x 10^8 CFU/ml for each test organism.

Susceptibility studies

Susceptibility studies of the various concentrations of ciprofloxacin alone, chloroquine alone and a combination of different concentration of ciprofloxacin and chloroquine were performed by a standard dilution technique with Mueller Hinton Agar (Lab M), (Washington and Sutter, 1980) using 100µl (2 x 10^7, 1 x 10^7 and 1 x 10^7 respectively for *P. aeruginosa, K. pneumoniae* and *E. coli* of overnight broth culture of each bacterial species with incubation at 37^{o}C for 24 hours. The zones of complete inhibition (as judged by the unaided eye) were measured to the nearest whole millimeter. The determinations were carried out in duplicates.

Statistical Analysis

The average sizes of the zones of inhibition corresponding to ciprofloxacin alone and a combination of different concentration of ciprofloxacin and chloroquine phosphate were statistically analyzed using paired t-test at 95% confidence interval. A $p \leq 0.05$ was considered significant.

RESULTS

The quality assessment of the pure ciprofloxacin hydrochloride and chloroquine phosphate gave 101.5%w/v and 100.9%w/v respectively which conform to official specifications for the pure drugs. The result of the biochemical reactions confirms the identifications of *Pseudomonas aeruginosa (PA), Klebsiella Pneumoniae (KP)* and *E. coli* (EC).

Three strains of *P. aeruginosa* (PA_1, PA_2 and PA_4) and *K. pneumoniae* (KP) clinical isolates were sensitive to different concentrations of ciprofloxacin alone as well as its combination with different concentrations of chloroquine (Figure 1- 4). However, one strain of *P. aeruginosa* (PA_3) was sensitive to ciprofloxacin alone but lost its sensitivity when ciprofloxacin was combined with chloroquine. Expectedly, none of the isolates was sensitive to chloroquine phosphate even at the highest concentration of 5µg/ml.

With the exception of PA_4 which had a MIC of 1.0 µg/ml of ciprofloxacin, the other three isolates of *P. aeruginosa* and the one strain of *K. pneumoniae* were susceptible at ≤0.5µg/ml of ciprofloxacin as determined by the values of the zones of inhibitions. However, the two strains of *E. coli* (EC_1 and EC_2) isolates were neither sensitive to the two drugs separately nor their combinations.

The statistical evaluation of the obtained result using the paired t-test at 95% confidence interval showed that chloroquine does not significantly affect the antibacterial activity of ciprofloxacin on PA_1 and PA_4 strains of *P. aeruginosa* and the strain of *K. pneumoniae* (KP) ($p > 0.05$). However, a significant enhancement of activity was observed on the PA_2 strain of *P. aeruginosa* ($p < 0.05$).

DISCUSSION

The two strains of *E. coli* used in this study were not susceptible to the different concentration of ciprofloxacin, chloroquine or their combinations. On the other hand, three of the four strains of *P. aeruginosa*

and the one strain of *K. pneumoniae* showed varying degree of susceptibility to different concentrations of ciprofloxacin and its combinations with chloroquine. However, the antibacterial activities of ciprofloxacin against one of the strains of *P. aeruginosa* (PA_3) were lost completely in the presence of chloroquine, even at the lowest concentrations of 1ug/ml of chloroquine. This may indicate a pharmacodynamic interaction of chloroquine on the activity of ciprofloxacin with respect to this particular strain of *P. aeruginosa*.

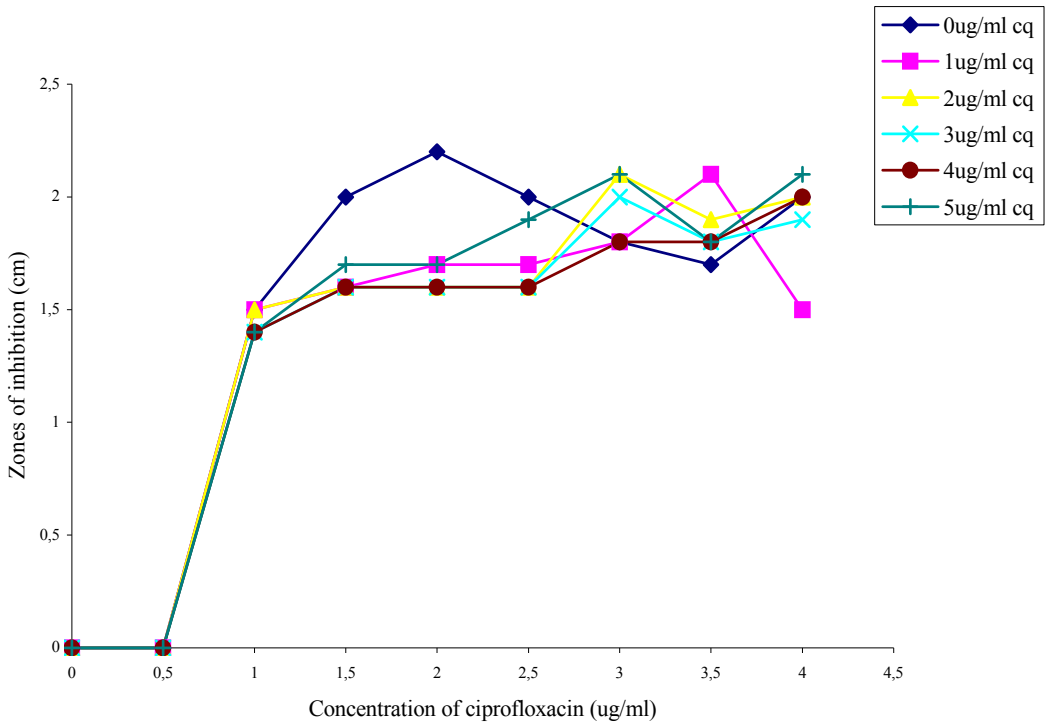

Figure 3. Zones of inhibitions of the pure ciprofloxacin and in combination with chloroquine against P. aeruginosa (PA_4)

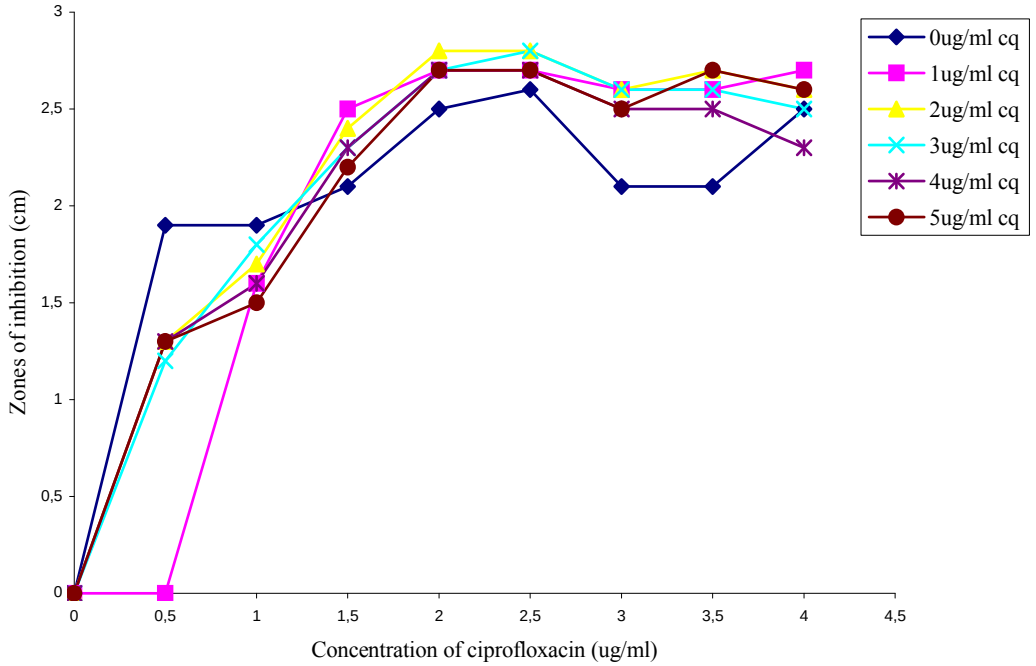

Figure 4. Zones of inhibitions of the pure ciprofloxacin and in combination with chloroquine against K. pneumoniae (KP)

Although, the antibacterial activities of ciprofloxacin was increased in the presence of chloroquine, the observed susceptibility obtained in this study did not indicate any significant effect of chloroquine on the different concentrations of ciprofloxacin for most of the strain of organisms studied.

The results obtained from the agar diffusion assay with ciprofloxacin hydrochloride (0.5 – 4.0µg/ml) alone and its combination with chloroquine phosphate (1.0-5.0µg/ml) on PA_1,PA_2, PA_4, and KP, when statistically analyzed using a 2-tailed student t-test, indicated that there were no significant differences between the inhibition zones produced by ciprofloxacin alone at 0.5-4.0µg/ml and its combination with chloroquine phosphate at 1.0-5.0µg/ml ($p>0.05$). This indicated an absence of a significant pharmacodynamic interaction between ciprofloxacin hydrochloride and chloroquine phosphate at the concentration ranges of 0.5–4.0µg/ml and 1.0-5.0µg/ml respectively on the strains of PA_1, PA_4 and KP used in vitro. This shows that chloroquine may not affect the antibacterial activity of ciprofloxacin on these isolates significantly.

On the other hand, a significant enhancement of in vitro antibacterial activities of ciprofloxacin hydrochloride by chloroquine phosphate at the concentration ranges of 0.5-4.0µg/ml and 1.0-5.0µg/ml respectively on PA_2 isolate was observed. A significant difference between the zones of inhibitions produced by ciprofloxacin hydrochloride alone at 0.5-4.0µg/ml and its combination with chloroquine phosphate at 1.0-5.0µg/ml ($p<0.05$) was observed.

Extrapolating the result of this study to in vivo conditions indicates that the earlier report by Rollof and Vinge (1993), on the impairment of elimination of ciprofloxacin in the presence of chloroquine may not be clinically significant with respect to the antibacterial activity of ciprofloxacin on the strains PA_1, PA_4 as well as on the KP organisms used in this study. However, a clinically significant loss of antibacterial activity will be expected with the strain PA_3 of the *P. aeruginosa*.

Earlier reports on the effect of chloroquine on the antibacterial activities of some antibacterial agents indicated enhancement of antibacterial activity of the agents. Crowle and May (1990) reported an enhancement of antibacterial activity of streptomycin on *Mycobaterium tuberculosis* in the presence of chloroquine. Similarly, chloroquine competitively inhibits the accumulation and activity of iron in yeast cells resulting in death of yeast cells. However, no report has been made on the effect of choroquine on the antibacterial activities of drugs on the microorganisms used in this study. The resistance of the clinical isolate of *E. coli* to ciprofloxacin observed in this study corroborates earlier reports on the possibility of resistance of uropathogens such as *E. coli* to ciprofloxacin which is encountered in hospital management of urinary tract infections (Mombelli et al 1999).

The results of the antibacterial evaluation of the drug combinations showed that no generalized statement could be made on the effect of chloroquine on the antibacterial activities of ciprofloxacin. An inhibition, enhancement or lack of effect observed depends on type and strain of microorganism involved.

In view of the result obtained in this study, further studies is hereby recommended to fully elucidate as well as probe into the clinical implications of co-administration of the two drugs during therapy. However, the results obtained in this study proposes that the administration of chloroquine during the management of bacterial infection using ciprofloxacin should be evaluated based on the individual merits and/or demerits.

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