Additive effects of ciprofloxacin on the in-vitro activity of chloroquine against a clinical isolate of *Plasmodium falciparum*

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As chloroquine and ciprofloxacin each possess substantial inhibitory activity against the schizonts of *Plasmodium falciparum*, it seems possible that a combination of the two drugs may be clinically useful. The effects on the erythrocytic stages of *P. falciparum* of combined treatment with chloroquine and ciprofloxacin were therefore evaluated *in vitro*, using the World Health Organization's standardized micro test. When used alone, the median inhibitory concentration (IC$_{50}$) of chloroquine against the schizonts in the assay mixtures was found to be 7.75 µg/ml, whereas the corresponding value for ciprofloxacin was markedly lower, at 3.35 µg/ml. When they were used together, however, there was marked and statistically significant mutual enhancement of schizont inhibition by the two drugs, indicating that a chloroquine–ciprofloxacin combination may be useful clinically, in the treatment and management of *P. falciparum* malaria.

In the treatment and control of human infectious diseases, the concurrent use of two or more pharmacological agents is a common clinical practice. Multiple drugs are often used simply because one drug is better against one aspect of the disease than another, but the use of drug combinations may sometimes result in an unexpected change in the overall efficacy of treatment, as a result of drug–drug interactions (Wilson *et al.*, 1971). Ampicillin and phenothiazine, for example, can indirectly enhance the antimalarial activity of chloroquine (Kozubek *et al.*, 2000). In contrast, the antibacterial activity of ciprofloxacin (CPF) may be lowered when iron is co-administered, because the iron hinders the absorption of the drug (Gorrod and Beckett, 1978). Other combinations may increase the frequency of adverse effects or even trigger adverse effects that are not observed when each drug is used alone. The co-administration of chlorpromazine with chloroquine (CQ), for example, may cause foetal retinopathy (Agarwal *et al.*, 2000).

In areas of tropical Africa where malaria is endemic, many patients who present with malaria have concurrent bacterial infections and are therefore treated with both an antibiotic and an antimalarial drug. The antibiotic is often CPF which, thanks to its high efficacy against a wide range of bacterial infections (Alghasha and Nahata, 2000; Talan *et al.*, 2000), is the most frequently used of the fluoroquinolones (Gupta *et al.*, 1999). Despite the growing problem of parasites that are resistant to the drug, the antimalarial used for first-line treatment is still often CQ, which is cheaper that any other antimalarial agent (Foster, 1994). The co-administration of CPF and

CQ in the management of malarial infection complicated by a bacterial infection is therefore not uncommon. In a study in Nigeria, this combination was found to be the one most frequently used in the treatment of malaria cases who have urinary-tract or upper respiratory-tract infections (O. O. Jonathan, unpubl. obs.). Curiously, CPF has been found to have some intrinsic antimalarial activity *in vitro*, with a reported median inhibitory concentration (IC_{50}) against *Plasmodium falciparum* of <10 μg/ml (Divo *et al.*, 1988; Mahmoudi *et al.*, 2003). This activity may explain why, at least in in-vitro assays against *P. falciparum*, tetracycline–CPF was found to be more effective than tetracycline alone, although the addition of CPF to fosmidomycin or novobiocin gave no significant benefit (Wiesner *et al.*, 2002). In their in-vivo study of the co-administration of CPF and CQ, Rolloff and Vinge (1993) reported some worrying neurological adverse effects, but the patients affected had also taken non-steroidal anti-inflammatory drugs. It seems possible that, since CPF has antimalarial as well as antimicrobial activity, a CQ–CPF combination may be a particularly good choice for the treatment of malaria cases with concurrent bacterial infections. The aim of the present, in-vitro study was to determine if use of this combination, against the erythrocytic stages of a clinical isolate of *P. falciparum*, led to a synergistic effect or to a decrease in antimalarial activity (compared with that predicted from the activities of each drug when used alone).

MATERIALS AND METHODS

Materials
The chloroquine phosphate and ciprofloxacin hydrochloride used were gifts from Bond Chemical Industries (Awe, Nigeria) and Gemini Pharmaceutical Industry (Lagos, Nigeria), respectively. A clinical isolate of *P. falciparum* and blood components were obtained from the haematology unit at the University College Hospital in Ibadan, Nigeria. The parasites were cultured using the standard method described by Trager and Jensen (1976) and a 'complete' medium consisting of RPMI 1640 supplemented with 25 mM HEPES, 0.2% (w/v) $NaHCO_3$, 0.005% (w/v) hypoxanthine and 10% (v/v) human O^+ serum. The medium was sterilized by filtration and stored at -20°C until required.

Measuring Antimalarial Activity *in Vitro*
The first series of experiments was designed to explore the ability of CQ or CPF to inhibit schizont formation by the *P. falciparum* isolate *in vitro*, using a slight modification (Wernsdorfer, 1980) of the World Health Organization's standardized, in-vitro, micro test (Rieckman *et al.*, 1978). All of these tests were performed in duplicate, with a separate plate for each replicate.

The ranges of drug concentrations tested — 1.0–7.0 μg/ml for CQ and 0.5–4.0 μg/ml for CPF — were based on the peak plasma concentrations (C_{max}) reported for CQ (0.98–2.90 μg/ml, with an erythrocyte-to-plasma concentration ratio of 3–4; Edwards *et al.*, 1988) and CPF (2.5 μg/ml; Watt *et al.*, 1991). Stock 0.01% (w/v) solutions of the two drugs were prepared in sterile distilled water and then diluted with complete medium, in the microtitre plates used for the tests, to give 50 μl drug solution in medium (or, for one control well, 50 μl of drug-free medium)/well. An equal volume of parasite culture, at 10% haematocrit and 0.5% parasitaemia, was then added to each well, before the plates were incubated in a micro-aerophilic atmosphere, in a sterile candle jar, at 37.5°C for 36 h. After this incubation, thin and thick smears of the cells in each well were prepared, Giemsa-stained and examined under a light microscope. For each well, the number of mature schizonts/200 parasites was counted and then expressed as a percentage of the corresponding value for the drug-free control well.

Linear extrapolations of these percentages (Mahmoudi *et al.*, 2003) were then used to estimate the drug concentration inhibiting 50% of the schizont maturation (IC_{50}).

DRUG INTERACTION
The first series of experiments was then repeated but with both drugs present in the wells, each drug being tested at the concentrations investigated previously. The fractional inhibitory concentrations (FIC) of CQ were determined, for each CPF concentration tested, as the ratio of the IC_{50} when the CQ was used in combination to the IC_{50} of the CQ when used alone. The FIC of CPF, for each CQ concentration tested, were similarly determined. An isobologram was constructed, by plotting, for each drug combination, the IC_{50} of one drug against the IC_{50} of the other (Wiesner *et al.*, 2002).

Statistical Analysis

A linear regression model was used to summarize the concentration–effect relationship (Mahmoudi *et al.*, 2003). The significance of the drug–drug interaction was evaluated using Student's *t*-tests, with a *P*-value of <0.05 taken as indication of a statistically significant difference.

RESULTS AND DISCUSSION

Monotherapy

When each drug was used alone, CQ was found to have an IC_{50} of 7.75 µg/ml and CPF an IC_{50} of 3.35 µg/ml. As expected (Mahmoudi *et al.*, 2003), the percentage inhibition of schizont development by each drug was found to be linearly related to the logarithm of the drug concentration.

The IC_{50} recorded for CPF corroborates earlier reports on the effects of CPF on *P. falciparum in vitro* (Wiesner *et al.*, 2002; Mahmoudi *et al.*, 2003). Although this IC_{50} is slightly above CPF's reported C_{max}

(2.5 µg/ml), the easy diffusion of the drug across the erythrocytic membrane means that the intra-erythrocytic concentrations of CPF are typically 1.5-fold higher than its plasma levels (Watt *et al.*, 1991). It is therefore possible for CPF to reach a concentration of at least 3.35 µg/ml (the IC_{50} recorded in the present study, when the drug was used alone) within the erythrocytes of a treated patient. The IC_{50} recorded for CPF in the present study is, however, markedly lower than the values reported when other *P. falciparum* strains were used as the targets. The corresponding IC_{50} recorded for the CQ-sensitive 3D7 strain (9.2 µg/ml; Mahmoudi *et al.*, 2003), two other CQ-sensitive isolates (5.3 and 6.4 µg/ml; Tripathi *et al.*, 1993; Weisner *et al.*, 2002) and a CQ-resistant strain (5.6–9.6 µg/ml; Watt *et al.*, 1991), for example, were all higher.

As with CPF, the IC_{50} of CQ when used alone (7.75 µg/ml) was higher than the reported plasma concentrations in treated patients (0.98–2.90 µg/ml; Edwards *et al.*, 1988) but intra-erythrocytic concentrations of the drug in treated patients, which are 3- to 4-fold higher than those in the surrounding plasma (Edwards *et al.*, 1988), could often reach the IC_{50}.

CQ and CPF in Combination

The effects on schizont maturation of all the various combinations of CQ and CPF investigated are illustrated in Figures 1 and 2. In general, the IC_{50} of each drug was lower when that drug was used in combination than when it was used alone, and the FIC for one drug decreased as the concentration of the other drug in the combination increased (Tables 1 and 2), indicating some interaction between the two drugs.

In the isobologram that was constructed (Fig. 3), the data-points fall more-or-less on a straight line, indicating that the drug–drug interaction observed was simply additive, and not synergistic or antagonistic (Wiesner *et al.*, 2002). The enhancements of the
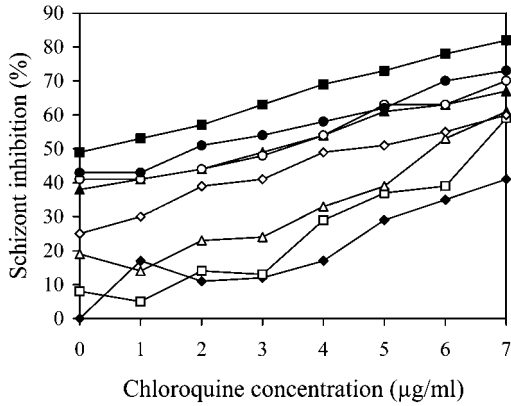
FIG. 1. Effect of ciprofloxacin, at 0.0 (◆), 0.5 (□), 1.0 (△), 1.5 (◇), 2.0 (▲), 2.5 (○), 3.0 (●) or 4.0 (■) μg/ml, on the inhibition by chloroquine of schizont production in cultures of *Plasmodium falciparum*.
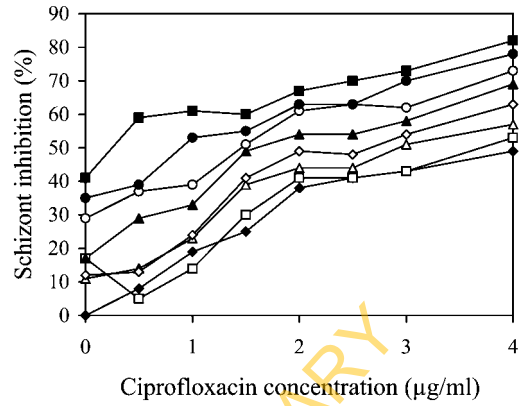

FIG. 2. Effect of chloroquine, at 0.0 (◆), 1 (□), 2 (△), 3 (◇), 4 (▲), 5 (○), 6 (●) or 7 (■) μg/ml, on the inhibition by ciprofloxacin of schizont production in cultures of *Plasmodium falciparum*.

antimalarial activity, of CPF by CQ (as the concentration of CPF was increased) and of CQ by CPF (as the concentration of CQ was increased), were statistically significant ($P<0.05$ for each).

When Divo *et al.* (1988) investigated the activities of combinations of CPF and tetracycline against *P. falciparum*, they observed sum FIC, of 0.79–0.93, that indicated modest additive effects. The addition of CPF to novobiocin (Divo *et al.*, 1988) or fosmidomycin (Wiesner *et al.*, 2002), however, gave no benefit in terms of antimalarial activity. Although Rollof and Vinge (1993) observed neurological adverse effects in patients given CQ–CPF, it was unclear whether the combination was the direct cause of the adverse effects because the affected patients had also received non-steroidal anti-inflammatory drugs.

The mutual enhancement of antimalarial activity seen when CQ and CPF are used

TABLE 1. *The median inhibitory concentrations (IC$_{50}$) and fractional inhibitory concentrations (FIC) of chloroquine, measured in vitro against a clinical isolate of* Plasmodium falciparum, *in the presence of various concentrations of ciprofloxacin*

Chloroquine value	Ciprofloxacin concentration (μg/ml)							
	0.0	0.5	1.0	1.5	2.0	2.5	3.0	4.0
IC$_{50}$ (μg/ml)	7.75	7.10	5.71	4.10	3.42	3.37	2.20	0.10
FIC	–	0.92	0.74	0.53	0.44	0.43	0.28	0.01

TABLE 2. *The median inhibitory concentrations (IC$_{50}$) and fractional inhibitory concentrations (FIC) of ciprofloxacin, measured in vitro against a clinical isolate of* Plasmodium falciparum, *in the presence of various concentrations of chloroquine*

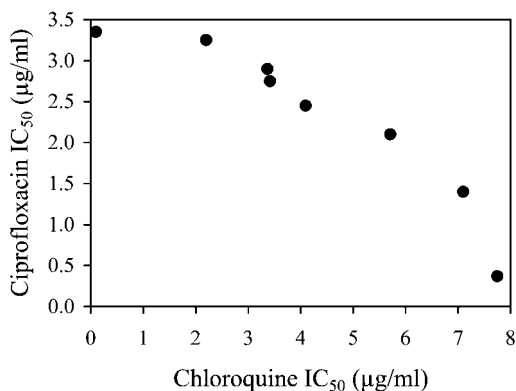| Ciprofloxacin value	Chloroquine concentration (μg/ml)							
	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0
IC$_{50}$ (μg/ml)	3.35	3.25	2.90	2.75	2.45	2.10	1.40	0.37
FIC	–	0.97	0.87	0.82	0.73	0.63	0.42	0.11

FIG. 3. An isobologram for the interaction of ciprofloxacin with chloroquine, in which the median inhibitory concentration (IC_{50}) of one drug is plotted against the corresponding value for the other drug, for several different combinations of the two drugs.

together indicates that a CQ–CPF combination may be a particularly good choice in the treatment of cases of CQ-sensitive or even CQ-resistant (Mahmoudi *et al.*, 2003) *P. falciparum* malaria who have bacterial infections. Curiously, when Yeo and Rieckmann (1994) tested CPF against a strain of *P. falciparum in vitro*, they found that the minimum inhibitory concentration of the drug fell from 28.1 μg/ml with a 48-h incubation to just 2.8 μg/ml with an incubation of 144 h. Such a delayed-kill effect of CPF may mean that the drug would not be useful in monotherapy against *P. falciparum* but may be useful both in chemoprophylaxis and in combined treatment with CQ, complimenting the rapid action of the latter drug.

Although the efficacy of a combination of CQ and CPF needs to be confirmed *in vivo*, initially in experimental models, the present results are encouraging and indicate that this combination may have considerable value in the treatment of *P. falciparum* malaria, with or without concurrent bacterial infection.

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