Asian Journal of Pharmaceutical and Clinical Research
Vol.2 Issue 4, October-December 2009 ISSN 0974-2441

Research Article

IN VIVO EVALUATION OF THE CO-ADMINISTRATION OF CHLOROQUINE AND CIPROFLOXACIN ON *P. BERGHEI* INFECTED ALBINO MICE

*OLAYEMI M. ADEGBOLAGUN, FATAI O. BALOGUN Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ibadan, Ibadan, e-mail:duplag03@yahoo.com
SAMUEL E. UHUNWANGHO Department of Biochemistry, Faculty of Biological Sciences, Igbinedion University, Okada, via Benin, Edo State, Nigeria

ABSTRACT
Previous reports have revealed antiparasitic activity of ciprofloxacin (CIP) as well as its additive effect on the activity of chloroquine (CQ) *in vitro*. This study was aimed at determining the clinical implications of the in-vivo administration of the two drugs. Male and female albino mice were randomly divided into ten groups of four animals each including a negative group without infection and a positive control infected with chloroquine – sensitive NK-65 strain of P. berghei but without treatment. The remaining groups were administered pure CQ at 12.5 and 25mg/Kg and CIP at 5 and 10mg/Kg as well as combination of the two concentrations. The biological evaluation involved monitoring the parasitaemia level over a 72hour period as well as the effect on the sperm motility and morphology and the liver enzymes [Glutamic-oxaloacetate transaminase (GOT) and Glutamic-pyruvic transaminase (GPT)]. The reduction in parasitaemia for the combination of the two drugs was not significantly different from those of the individual drugs ($p>0.05$). However, a significant reduction in sperm motility as well as a significant increase in percentage aberration was observed in the presence of infection when compared with healthy state ($p< 0.05$). On the other hand, a non-significant difference in sperm motility was observed with the combination of the two drugs at the two concentrations. A significant decrease in the percentage aberration was obtained with the combination of the two drugs when compared with the presence of infection (positive control) which was not significantly different from that of the absence of infection (negative control). The lower concentration combinations of the two drugs gave significantly lower values than that of the higher concentration combinations ($p<0.05$). A non-significant difference in the GOT and GPT activity was obtained for the combination of the two drugs at the different combinations ($p>0.05$). The obtained result suggests that the combination of CQ and CIP at the two concentrations at a single dose administration possesses rapid parasite clearance as well as relative safety on the reproductive system and the liver. Thus the combination may be of clinical benefit in the management of malaria.

KEYWORDS Chloroquine, ciprofloxacin, chloroquine-sensitive *P. berghei*,

INTRODUCTION

The co-administration of two or more drugs may result in drug-drug interaction. A potential drug interaction refers to the possibility of a drug altering the intensity of the pharmacological effect of another drug taken concurrently resulting in either increased or decreased effect of one or both drugs or the existence of a new effect that is not peculiar to or seen with either of the drugs given alone[1].

Malaria is one of the most serious protozoa infections, which affect human and is caused by infection of any of Plasmodium species[2]. Chloroquine, 7-chloro4-(4-diethylamine-1-methylbuthyl amino) quinoline, is the drug of choice for the treatment of infections acquired in the areas where the strains of Plasmodium are still sensitive to the drug. Ciprofloxacin, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7piperazinylquinoline-3-carboxylic acid, on the other hand, is a fluoroquinolone anti-bacterial agent with a wide spectrum of activity which have been reported to have antiparasitic activity on various strains of *P. falciparum*[3,4].

The frequent association of malaria with other infections like catarrh, pneumonia, diarrhoea, etc. has necessitated the co-administration of antimalarials with a number of other therapeutic agents such as antibiotics, analgesics, antiallergic etc. Co-administration of antimalarial agents

especially chloroquine with antibiotics such as ciprofloxacin in not uncommon in malaria endemic areas like Africa, as such there could be interaction between the two drugs. The administration of chloroquine with some antibiotics have been reported; decrease ampicillin and cloxacillin bioavailability was reported when administered separately[5,6]. On the other hand, a significant additive effect was reported with in vitro combination of ciprofloxacin and chloroquine on a clinical isolate of P. falciparum[7].

The reported additive effect of the in vitro co-administration of ciprofloxacin on chloroquine led to this study which was aimed at determining the clinical implications of the in-vivo administration of the two drugs.

MATERIALS AND METHODS

Chemicals

Ciprofloxacin hydrochloride powder was obtained from Gemini Chemical Company, Lagos, Nigeria, while chloroquine phosphate powder was obtained from Bond Chemical Industry, Aawe, Oyo State, Nigeria. Chloroquine – sensitive NK-65 strain of P. berghei was obtained from Dr. A. Ademowo of Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University College Hospital, Ibadan, Nigeria. Reagents used include *g*lacial acetic acid, perchloric acid, acetic anhydride, potassium hydrogen phthalate, hydrochloric acid, crystal violet powder, sodium chloride, Oil Immersion, Giemsa stain solution (pH 7.2), Negrosin-eosin solution, methanol and chloroform. All the reagents were of analytical grade. Glutamic-Pyruvic Transaminase (GPT) and Glutamic-Oxaloacetate Transaminase (GOT) were from Randox Lab. Ltd.

Laboratory animals

Male and female albino mice weighing between 17-20g obtained from the Animal Centre of the Nigerian Institute of Medical research (NIMR), Yaba Lagos, Nigeria were used for the study. The animals were kept in ventilated metal cages under standard conditions (Temperature: 28-30^{o}C, Photoperiod: 12h natural light and 12h dark), fed with mouse cubes (Ladokun Feeds, Ltd, Nigeria) and water *ad libitum.* The animals were stabilized over a period of two weeks. The study was conducted in accordance with the recommendations from the declaration of Helsinki on guiding principles in care and use of animals.

Induction of Infection

A donor mouse was infected with cryopreserved chloroquine-sensitive parasite and thereafter allowed to developed parasitaemia. The donor mouse was sacrificed when the percentage parasitaemia was greater than 60%. 0.2ml of the blood in Acid Citrate Dextrose (ACD) collected by cardiac puncture was transferred into sample bottle; this was further diluted with 7.8ml of normal saline. 0.1ml of ACD was mixed with the blood of the donor mice to make up to 0.2ml suspension of the parasite, which was injected subcutaneously into the experimental mice except Group 1. The percentage parasitaemia was determined after the 5th day of parasite infection.

Experimental Design

Forty animals were randomly divided into ten groups of four animals each. Groups 1 and 2 were negative controls with Group 1 having healthy animals without infection, while Group 2 was infected with the parasite without

treatment. Groups 3- 10 were also infected with the parasite. Groups 3–6 were positive controls, with Groups 3 and 4 administered with chloroquine (12.5 and 25mg/Kg body weight respectively), while Groups 5 and 6 were administered ciprofloxacin (5 and 10mg/Kg body weight respectively). Groups 7 – 10 were the treatment groups, with groups 7 and 8 treated with a mixture containing 12.5 mg/Kg chloroquine and ciprofloxacin at (12.5 mg/Kg: 5 mg/Kg and 12.5 mg/Kg: 10 mg/Kg respectively). Groups 9 and 10 were treated with a mixture containing chloroquine and ciprofloxacin (25 mg/Kg: 5 mg/Kg and 25 mg/Kg: 10 mg/Kg respectively).

The drugs were administered orally. The animals were allowed free access to their feed and water before and throughout the period of the study.

Parasitaemia monitoring

Parasitaemia was determined at 0, 24, 48 and 72 hours after drug administration by obtaining blood from the caudal vein, using an earlier reported method[8].

Toxicological evaluation

The animals were sacrificed under chloroform anaesthesia; about 2ml of blood was collected by cardiac puncture using syringe and needle into bottles. The blood samples were centrifuged at 3000rpm to obtain the serum which was used for the liver enzyme activity assessment.

i. Sperm motility and morphology- The sperm motility and morphology was determined according to the earlier reported method[9].

ii. Liver Enzyme Activity Assessment- GOT and GPT activity assessment in the sera was determined using Ultraviolet spectrophotometer (Randox Lab. Ltd).

Statistical Analysis

Data are expressed as mean of four replicates ± S.D. Statistical analysis was carried out using Student's t-test and one-way analysis of variance (ANOVA) where necessary. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

A non-significant enhancement of CQ activity by 5mg Ciprofloxacin CIP at both concentrations of CQ used in this study was observed. The overall effect of both concentrations of Ciprofloxacin CIP (5 and 10mg/Kg) on the two concentrations of CQ by the end of the study at 72hours was found not to be significantly different from that of the pure CQ at the same concentrations ($p>0.05$), (TABLE 1, FIG. 1).

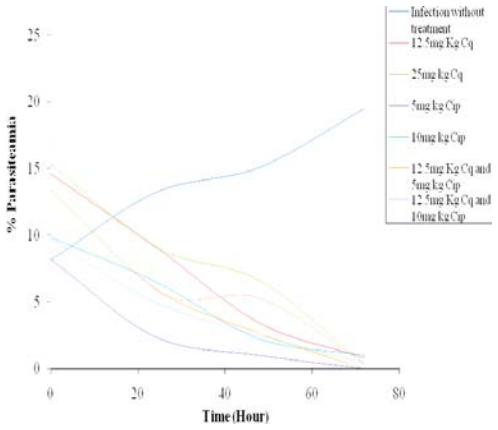

FIG.1.Percentage parasitaemia obtained after administration of pure chloroquine and ciprofloxacin and their combinations at different concentrations to *P. berghei* infected male albino rats.

CIP has been reported to possess antiparasitic effect against different strains of *P. falciparum*[3, 4]. An earlier report on the antiparasitic activity of CIP reported a delayed time effect which was found to largely dose-dependent with a progressive

increase in inhibitory effect by 72 and 96hours[3]. This is in agreement with the result obtained in this study with the 10mg/Kg CIP showing a delayed time in activity at 24hours which later increase significantly by 48 and 72hours. The result obtained in this study thus suggests that the combination of the lower concentrations of CIP and CQ, i.e. 5mg and 12.5mg/Kg respectively would show a rapid response initially which would on the overall elicit similar response as the combination of the two drugs at the higher concentrations by 72hours. The result of this study is however slightly different from the significant decrease in parasitaemia by rifampicin with high concentration of CQ[8]. Similarly, the in vitro combination of ciprofloxacin or azithromycin and CQ were reported to exhibit additive effect on CQ–sensitive *P. falciparum* isolates[7, 10].

A reduction in sperm motility was observed in the presence of infection in this study which is in agreement with the report of Raji et al (2006) on the decrease in sperm motility of mice infected with *P. berghei*[11]. This was further reduced by CQ and CIP (TABLE 2). A decrease in sperm motility by chemical agents has been reported by Baldessarini[12]. CQ and ciprofloxacin have been reported to cause a decrease and impairment in sperm motility[11, 13-17]. The obtained reduction in sperm motility obtained for the combination of the two drugs was not significantly different from those of the individual drugs and that of the infection without treatment (TABLE 2).

Table 1- Percentage parasitaemia obtained after administration of pure concentrations of chloroquine and ciprofloxacin and at different concentration combinations to *P. berghei* infected male albino rats.

GROUP	CONDITION	% PARASITEAMIA AT TIME (HOUR)			
		0	24	48	72
1	Without infection and treatment	-	-	-	-
2	Infection without treatment	8.14± 1.84	13.2±7.47	15.1±7.59	19.5 ± 7.21
3	12.5mg/Kg Cq	14.6 ± 6.2	9.15 ±3.89	3.40 ±1.98	0.8 ±0.28
4	25mg/kg Cq	15.36 ± 4.15	9.10 ±3.11	6.60 ±5.10	0.30 ±0.0
5	5mg/kg Cip	8.20 ±7.35	2.40 ±2.26	1.0 ±0.00	0.00
6	10mg/kg Cip	9.80 ±6.4	6.50 ±3.31	2.2 ±1.59	1.0 ±0.0
7	12.5mg/Kg Cq and 5mg/kg Cip	13.36 ±2.27	6.0 ±3.62	2.65 ±2.17	0.00
8	12.5mg/Kg Cq and 10mg/kg Cip	9.62 ±4.92	4.97 ±2.38	2.48 ±2.01	0.4 ±0.28
9	25mg/Kg Cq and 5mg/kg Cip	16.6 ±0.57	5.93 ±5.27	5.25 ±5.87	0.4 ±0.00
10	25mg/Kg Cq and 10mg/kg Cip	8.95 ±2.	7.55 ±2.90	2.65 ±0.92	0.00

ND – Not Determined

Sperm morphology evaluation was aimed at identifying the proportion of abnormal cells. The most common abnormality obtained in this study was ‘cut tail’ which is a secondary abnormality attributed to changes taking place during storage in the epididymis or beyond[18].

A significant increase in percentage aberration was observed in the presence of infection when compared with the absence of infection ($p<0.05$), (TABLE 2). However, a significant decrease in

percentage aberration was observed with pure CQ at 12.5 and 25mg/Kg as well as at 5mg/Kg CIP when compared with the presence of infection without treatment (positive control), while a non-significant reduction was observed at 10mg/Kg of CIP (TABLE 2). Previous studies have reported
antifertility effects of some antimalarial agents such as quinine, artemisinin, halofantrine and chloroquine[19-23]. The most common abnormality in morphology observed for artemisinin was curved mid-piece which is also a secondary and tertiary aberration, but this was associated with high dose and long duration of use at recommended dose[19]. Furthermore, halofantrine have been reported to induce formation of immature spermatocytes which would result in the reduction of viable sperms[20]. However, CIP have been reported not to have any effect on the sperm morphology of healthy male Wistar rats[14], although, recognizable histological damage associated with a mild decrease in testicular volume and sperm concentration on healthy rats have been reported[15]. There has not been any report on the effect of the combination of CIP and CQ on sperm motility and morphology.

The combination CIP and CQ at the concentrations used in this study gave an increase in the percentage aberration when compared with the pure CQ but the values obtained are similar to those obtained at the two concentrations of CIP used in this study. The percentage aberration obtained for groups 7 and 8, i.e. combination of 12.5mg CQ with 5 and 10mg/Kg of CIP were not significantly from that obtained

Table 2- Mean % sperm motility and morphology after administration of chloroquine and ciprofloxacin and their combinations at different concentrations to *P. berghei* infected male albino rats.

GROUP	CONDITION	SPERM MOTILITY (% MOTILITY)	% ABBERATION (%)
1	Without infection and treatment	>90	15.4
2	Infection without treatment	70 – 80	41.7
3	12.5mg/Kg Cq	50 - 60	21.4
4	25mg/kg Cq	60 - 68	29.4
5	5mg/kg Cip	60 – 70	28.6
6	10mg/kg Cip	60 – 70	35.7
7	12.5mg/Kg Cq and 5mg/kg Cip	65 – 75	27.8
8	12.5mg/Kg Cq and 10mg/kg Cip	70 – 80	33.3
9	25mg/Kg Cq and 5mg/kg Cip	70 – 80	38.3
10	25mg/Kg Cq and 10mg/kg Cip	ND	**ND**

for pure CIP at 5 and 10mg/Kg respectively.

This suggests that whatever effect the combination of the two drugs may have on the percentage aberration does not differ significantly from that obtained with the pure CIP at the two concentrations used in this study.

Glutamic oxaloacetate transaminase (GOT) is an enzyme present in liver, nervous tissue, skeletal muscle and heart, it catalyses the conversion of aspartate to oxaloacetate and glutamate in mitochondrion and cytosol. Membrane damages or necrosis release this enzyme into circulation. High levels of GOT in serum indicate liver damages due to toxicity and viral hepatitis as well as cardiac infections and muscle injury[24, 25]. Glutamic-pyruvic transaminase (GPT) is also a cytosolic and mitochondria enzyme but more specific to the liver and catalyses the conversion of alanine to pyruvate and glutamate. This enzyme when released into circulation in a similar manner and high levels also indicate hepatitis and liver damage[24, 25]. The liver enzyme assessment showed a non-significant difference in the GPT values obtained at different concentrations of the pure CQ and CIP when compared with the combinations of the two drugs. However, a non-significant increase in GOT activity was obtained in the presence of infection when compared with the control group without infection. GOT and GPT activity values obtained were higher at the higher concentrations of the two drugs when compared with their lower concentrations(TABLE 3).Generally, a non-significant difference in GOT activity was obtained for the combination of the two drugs when compared with that of the positive control group with infection and without treatment ($p>0.05$, ANOVA), (TABLE3). Similar non-significant reduction in GPT values was also obtained with the combination of the two drugs at the various combinations. The non-significant difference in the values of the two serum enzymes; GOT and GPT, in the presence of the infection and at the different concentrations of CQ is in agreement with an earlier report by Stemberger et al (1984)[21]. They reported absence of drug – associated liver damage with the evaluation of the activity of GOT and GPT activity in serum of humans and no such side effects with long time use of CQ in chemoprophylaxis on malaria.

On the other hand, another set of workers, Okonkwo et al (1997)[22] reported increase in the activity of GOT in mice which is in agreement with the obtained values at higher concentration of CQ at 25mg/Kg.

However, the high concentrations of CQ and CIP and their combinations may not constitute a risk to the liver function as the increase in GOT values is not significant. The increase in GOT level for CQ at the higher concentration is in agreement with earlier report on another antimalarial; halofantrine alone and in combination with other antibiotics such as minocycline and hygromicin[26]. Inclusion, the obtained results in this study suggests that the combination of CIP and CQ at the lower concentration of 5mg and 12.5mg/Kg possesses rapid parasite clearance rate along with relative safety on the reproductive system as observed by the non-significant effect on the sperm motility when compared with the disease state as well as significant reduction in the % aberration. Moreover, GOT and GPT activity values were not significantly different from those obtained with the control groups i.e. in the absence of infection and in the presence of infection without treatment. This suggests that this concentration combination constitute no risk to the liver function. Thus, the combination of CQ and CIP at concentrations of 5mg/Kg and 12.5mg/Kg respectively may be of clinical benefit in the management of P. falciparum infection since the *P. berghei* animal model used in this study has been shown to have similar genetic composition to the *P. falciparum* parasite.

ACKNOWLEDGEMENT

The authors acknowledge Gemini Pharmaceutical Industry, Lagos, Nigeria and Bond Chemical Industries, Ltd. Awe, Oyo – State, Nigeria for the provision of pure ciprofloxacin hydrochloride powder and chloroquine phosphate pure powder respectively used for the study. Similar thanks also go to Dr. A. A. Ademowo of Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University College Hospital, Ibadan, Nigeria, for the Chloroquine–sensitive NK-65 strain of *P. berghei.*

REFERENCES

1. Aguwa CN. (ed). Therapeutic Basis of Clinical Pharmacy in the Tropics. Macmillan Publishers, London, UK. 1996.
2. Martindale: The Extra Pharmacopoeia (30^{th} ed.): The Pharmaceutical Press, London. 1999.
3. Mahmoudi N, Liliane C, Franetich JF, Farhati, K, Silvie O, Eling W et al. In vitro activities of 25 Quinolones and Fluoroquinoloes against Liver and Blood Stage Plasmodium falciparum spp. Antimicrob. Agents Chemother 2003, 47(8): 2636 – 2639.
4. Yeo AE, Rieckmann KH. Prolonged exposure of *Plasmodium falciparum* to ciprofloxacin increase antimalarial activity. J. Parasitology 1994; 8: 158-160
5. Ali HM. Reduced Ampicillin bioavailability following oral co-administration with Chloroquine. J. Antimicrob. Chemother 1985; 15: 781-784.
6. Babalola CP, Fakeye TT, Olaniyi AA. Chloroquine reduces Urinary excretion of Cloxacillin when administered concurrently with Ampicillin-Cloxacillin

combination. Trop. J. of Pharm. Res 2003; 2(1): 169 – 173.

7. Kazzim OJ, Adegbolagun OM, Osho O, Anumudu CI. Additive effects of ciprofloxacin on the in vitro activity of chloroquine against a clinical isolate of Plasmodium falciparum. Ann of Trop Med & Para 2006; 100(7): 579-584.
8. Lo Jen HS, Sivachandra RM, Shukri A. Rifampicin antagonizes the effect of Chloroquine-Resistant Plasmodium berghei in mice. Japan J. Infect. Dis 2004; 57: 198 – 202.
9. Raji Y, Salman TY, Akinsomiye OS. Reproductive function in male rats treated with methanolic extract of Alstonia boonei stem bark. Afr. J. Biomed. Res 2004; 8(2): 105 – 111.
10. Ohrt C, Willingmyre GD**,** Lee P, Knirsch C, Milhous W. Assessment of Azithromycin in Combination with Other Antimalarial Drugs against *Plasmodium falciparum* In Vitro. Antimicrob. Agents Chemother 2002; 4(8): 2518-2524.
11. Raji Y, Akinsomiye OS, Azeez M. Impact of malaria parasite on reproductive indices of male mice. Reprod Med. Biol 2006; 5: 201 – 209.
12. Baldessarini RJ. Drugs and treatment of psychiatric disorders. In Gilman AG, Hardman JG and Limbird LE (Eds). *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. 10th Edition. McGraw, New York, 1996.
13. Adeeko AO, Dada OA. Chloroquine reduces the fertilizing capacity of Epididymal sperm in rats. Afr. J. Med. Sci 1998; 27: 63 - 68.
14. Marefat GN, Mahnaz H, Arash K. Effects of Ciprofloxacin on testis apoptosis and sperm parameters in rat. Iranian J. of Reproductive Medicine 2008; 6 (2): 71-76.
15. Adel RAA, Hamdy AAA. Adel MAA, Abdel-Aziz HA, Farid MAH. Adverse testicular effect of some quinolone members in rats. Pharmacological Research 2000; 41(2): 211-219.
16. Demir A, Turker P, Onol FF, Sirvance S, Findi K. Effect of experimentally induced *Escherichia coli* epididymo-orchitis and ciprofloxacin treatment on rat spermatogenesis. International Journal of Urology 2007; 14(3): 268 – 272.
17. Moss JA, Reed HCB. Fertility and Infertility in Domestic animals. In *Spermatozoa, Semen and Artificial insemination*. Laray JA. (Ed.), Brailiere Tindal, London, UK. 1979.
18. Criestensen AC. Leydig cell. In *Handbook of Physiology*. Greep PO and Astwoods EB. (Eds.), American Physiological Society, Washington DC, 1995.
19. Nwanjo HU, Iroagba II, Nnatuanya IN, Eze NA. Antifertility Activity of Dihydroartemisinin in Male Albino Rats. *The Internet Journal of Endocrinology* 2007; Volume 4, Number 1.
20. Didia BC, Ggigbi PS, Dapper DV. Preliminary study on the effect of Halofantrine hydrochloride on the testes of mature Wistar rats. Journal of Applied Sci. & Environmental Management 2002; 6(1): 45- 48.
21. Stemberger H, Leimer R, Wiedermann G. Tolerability of long-term prophylaxis with fansidar: A random double-blind study in Nigeria. Acta Trop 1984; 41(4): 391 – 394.
22. Okonkwo CA, Agomo PU, Mafe AG. A study of the hepatotoxicity of chloroquine (SN-7618) in mice. Nig. Quarterly J. Hospital Med 1997; 7: 183 – 187.
23. Obi E, Orisakwe OE, Asomugha LA. The hepatotoxic effect of halofantrine in Guinea pigs. Indian J. Pharmacol 2004; 36(5): 303 – 305.
24. Cornelius CE. Biochemical Evaluation of hepatic function in Dogs. J. Am. Hosp. Ass 1979; 15: 25 – 29.
25. Varley H, Gowenlock AH, Bell M. Practical Clinical Biochemistry. Vol. 1. 5th Edition. CBS Publishers and Distributors, India, 1991.
26. Bhat G, Jordan J (Jnr), Sokalski S. Minocycline – induced hepatitis with autoimmune features and neutropenia. J. Clin. Gastroenterol 1998; 27(1): 74 – 75.