Antimalaria Action of Antiretroviral Drugs on *Plasmodium berghei* in Mice

Akinwumi Akinyede,* Alade Akintonwa, Olufunsho Awodele, Sunday Olayemi, Ibrahim Oreagba, Charles Okany, Oluwagbemiga Aina, and Samuel Akindele

Department of Pharmacology, and Department of Hematology and Blood Transfusion, College of Medicine, University of Lagos, Lagos, Nigeria; Department of Biochemistry and Nutrition, Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria

Abstract. Malaria parasitemia enhances replication of human immunodeficiency virus. Antiretroviral drugs that possess antiplasmodial activity may reverse such an effect. Activity of the antiretroviral drugs lamivudine (L), zidovudine (Z), nevirapine (N), and stavudine (S) against *Plasmodium berghei* inoculated into 70 adult albino mice was investigated. Eight groups of five animals each were treated with different drugs as either curative or prophylactic regimens. These regimens were also given to four groups as L/Z/N or L/S/N. Z therapy alone and L/Z/N eliminated malaria parasites as follows: curative and prophylactic Z groups, mean $\pm$ SEM = 62,132.87 $\pm$ 22,816.1 parasites/ μ L and 62,474.85 $\pm$ 14,639.1 parasites/ μ L, respectively on day 4 and 0 parasites/ μ L on day 26; curative L/Z/N group, 31,583.53 $\pm$ 6,361.67 parasites/ μ L, and 0 parasites/ μ L (days 4 and 18, respectively); prophylactic L/Z/N group, 41,138.1 $\pm$ 3,528.03 parasites/ μ L, and 0 parasites/ μ L (days 4, and 20 respectively). Peters four-day suppressive values were 67–82.2%. Zidovudine or L/Z/N therapy may modify the epidemiology of malaria and therefore the pandemic of human immunodeficiency virus infection.

INTRODUCTION

The influence of human immunodeficiency virus (HIV) on malaria infection varies between areas of stable and unstable transmission.¹ In areas where malaria transmission is stable, HIV infection has been found to approximately double the risk of malaria parasitemia and clinical malaria in non-pregnant women.² Also, HIV infection is associated with an increased prevalence^{2–5} and density⁶ of parasitemia and an increased prevalence of clinical malaria^{2,3,7–9} particularly in patients with severe immunosuppression.^{2,3,7,10–12} In a cohort study in Uganda, the risk of clinical malaria was increased three times in patients with CD4 cell counts of 200–499 cells/ μ L and increased six times in patients with counts < 200 cells/ μ L than in patients with counts > 500 cells/ μ L; parasitemia also increased as the CD4 cell count decreased.²

The prevalence of severe malaria and mortality in areas of stable transmission was not affected by HIV infection in previous studies,^{13–16} but data from three recent studies challenge this finding.^{17–19} In a case-control study in Zambia, HIV infection was a significant risk factor for adults with severe malaria compared with controls with uncomplicated malaria (odds ratio = 12.6) and asymptomatic controls (odds ratio = 16.6).¹⁸ In Burkina Faso, a positive blood smear for malaria was an independent risk factor for death in HIV-infected patients.¹⁹ In a retrospective study in Senegal, malaria-related mortality was higher in HIV-infected patients than in HIV-uninfected patients (58% versus 19%; $P < 0.001$).¹⁷

In areas of unstable transmission, HIV infection is associated with an increased risk of severe malaria^{20–22} and death.^{22,23} In a prospective study in South Africa, HIV-infected patients had a four-fold increased risk of severe malaria, and the prevalence of severe malaria was the highest when the CD4 cell count was < 200 cells/ μ L. In subgroup analyses, HIV infection was associated with an increased risk of severe malaria, intensive care unit admission, and death in non-immune patients, but not in semi-immune patients.²¹ Data from two French studies indicated an association between

imported severe malaria and CD4 cell counts < 350 cells/ μ L in HIV-infected persons infected with malaria while traveling.^{24,25} Thus, in non-pregnant women without naturally acquired immunity, HIV infection is associated with an increased risk of severe malaria and death. In eastern and southern Africa, where HIV prevalence is near 30%, it is estimated that approximately 25–33% of clinical malaria in adults can be accounted for by HIV.²⁶

Malaria parasitemia also enhances the HIV replication.^{2,26–29} The co-existence of these two disease entities therefore potentiates their spread and is of public health importance. Against this background, any drug used in the therapy of HIV that also has an antimalarial effect will reduce the parasite biomass, parasite-induced HIV replication will be retarded, and ultimately the spread of HIV will be curtailed. Antiretroviral drugs of the class protease inhibitors have been proven to possess antimalarial activity,³⁰ these protease inhibitors include saquinavir, ritonavir and indinavir. However, protease inhibitors are not commonly used in malaria-endemic regions of sub-Saharan Africa. Lamivudine, zidovudine, and nevirapine and until recently, stavudine, are usually prescribed. Lamivudine, zidovudine, and nevirapine form the first-line therapy prescribed together as a triple combination in the region. There is paucity of information on the effects of this therapy on *Plasmodium* infection. Therefore, we assessed the antimalarial effect of lamivudine, zidovudine, nevirapine, and stavudine to further elucidate the interaction between malaria and HIV and provide more tools for the control of malaria and HIV.

MATERIALS AND METHODS

Adult albino mice weighing 15–25 grams were housed in groups of 5 animals in cages constructed with stainless steel and plastic materials. Maintenance and care of experimental animals complied with the Nigeria Institute of Medical Research guidelines for the use of experimental animals. The animals were kept in hygienic conditions in the animal house of the Nigerian Institute of Medical Research, Yaba, Lagos, where experiments were conducted. The animals were fed a high-quality mice diet and given water *ad libitum*. The NK 65 strain of the malaria parasite *Plasmodium berghei*, which is sensitive to chloroquine, was obtained from

*Address correspondence to Akinwumi Akinyede, Department of Pharmacology, College of Medicine, University of Lagos, PMB 12003, Lagos, Nigeria. E-mail: a_akinyede@yahoo.co.uk

Nigerian Institute of Medical Research, Yaba, Lagos. Drugs used were obtained from Aurobindo Pharmaceuticals Ltd., Hyderabad, India.

Method for determining curative and prophylactic effects of antiretroviral drugs on *P. berghei*. The experimental procedure for determination of parasitemia levels involved 14 groups of 5 animals each. Animals in group I were infected with *P. berghei* and distilled water was administered to these animals orally throughout the duration of the experiment. The parasitemia level was then monitored. Animals in group II were infected with *P. berghei*, and a therapeutic dose of chloroquine, 10 mg/kg once a day, was administered orally on the fourth and fifth days post-parasite infection, and 5 mg/kg was administered on the sixth day. Animals in group II were also used as controls for curative and prophylactic studies. Animals in the remaining groups (III–XIV) were also infected with *P. berghei*, and antiretroviral drugs were administered either on curative or prophylactic basis. Curative regimens refer to administration of drug therapy after the mice were infected with *P. berghei*, and prophylactic therapy was given by dosing the mice before infection. Doses of drugs used in this experiment were extrapolated from those normally prescribed to patients in the HIV clinic on the basis of weight of the animals used.

Administration of curative drug regimens. Animals in groups III, IV, V, and VI received therapeutic doses of lamivudine, zidovudine, nevirapine, and stavudine at 4.3 mg/kg, 8.6 mg/kg, 5.7 mg/kg, and 0.9 mg/kg, respectively, orally once a day over a 23-day period starting from the fourth day post-infection. Animals in groups VII and VIII received therapeutic doses of triple combinations of lamivudine, zidovudine, and nevirapine (LZN) and lamivudine, stavudine, nevirapine (LSN), respectively, equal to the single doses given orally once a day over a 23-day period also starting from the fourth day post-infection.

Administration of prophylactic regimens. Animals in groups IX, X, XI, and XII received therapeutic doses of lamivudine, zidovudine, nevirapine, and stavudine at 4.3 mg/kg, 8.6 mg/kg, 5.7 mg/kg, and 0.9 mg/kg, respectively, orally once a day starting 14 days before inoculation with *P. berghei*. Animals in groups XIII and XIV received therapeutic doses of triple combinations of LZN and LSN, respectively, equal to the single doses given orally once a day starting 14 days before inoculation with *P. berghei*. The prophylactic regimen in all cases continued until the 26th day post-infection.

Four-day suppressive test. The Peters four-day suppressive test against *P. berghei* infection was used.³¹ After three hours

of infection with parasites, the mice were randomly assigned into six treatment groups. One group, which was used as a negative control, received distilled water orally throughout the duration of the experiment, one group received chloroquine at 10 mg/kg, 3 groups received lamivudine, zidovudine, and nevirapine, at 4.3 mg/kg, 8.6 mg/kg, and 5.7 mg/kg, respectively, and the remaining group received a therapeutic dose of a triple combination of lamivudine, zidovudine, and nevirapine, respectively, equal to the single doses. All drugs were administered orally once a day for four consecutive days. This test was begun some months after the curative and prophylactic effects of the antiretroviral drugs were studied, by which time use of stavudine in HIV clinics in Nigeria had been discontinued because of cases of lactic acidosis associated with stavudine. Every effort at obtaining this drug failed. Thus, stavudine was omitted in the four-day suppressive test. The percentage suppression of parasitemia was calculated by using the formula % suppression = average % parasitemia in negative control – average % parasitemia in test group $\times$ 100/average % parasitemia in negative controls.

Inoculation of mice with malaria parasites. Animals were infected with parasitized blood obtained by snipping the tip of the tail of a previously infected mouse. Approximately 0.1 mL of infected blood (3–4 drops) was diluted in 0.9 mL of normal saline (0.9% NaCl). Mice were inoculated intraperitoneally with 0.1 mL of parasitized saline suspension containing approximately 1×10^7 parasites. Development of parasitemia was monitored by microscopic examination according to the method of Shida and others.³² Infected erythrocytes were counted by using the formula³³ malaria parasite density/ μ L of blood = no. parasites counted $\times$ 8,000/200 leukocytes. Average malaria parasite clearance/day was calculated by dividing the malaria parasite density on day 4 (initial malaria parasite density) by the number of days over which these parasites were completely cleared from the animals' blood specimen after administration of each drug therapy. Thus, average malaria parasite clearance/day = malaria parasite density on day 4 post-inoculation/no. days required to clear parasites completely.

The parasite reduction ratio (PRR) for the mice receiving a curative regimen was determined by calculating the ratio of the parasite count before treatment (P_0) to the count at 48 hours (P_2)³⁴ ($PRR = P_0/P_2$). Thus, PRR was not calculated for the animals on prophylactic regimen because contrary to the standard procedure, these animals were receiving antiretroviral drugs before infection with malaria parasites was done, and

TABLE 1

Effect of curative lamivudine, zidovudine, nevirapine, and stavudine and combination therapies on *Plasmodium berghei* infection in adult albino mice

Drug group	Parasite densities/ μ L of blood sampled on different days*				
	Day 4	Day 6	Day 8	Day 20	Day 26
I (distilled water)	67,794.5 $\pm$ 9,616.0	71,866.25 $\pm$ 7,418.26	(1D) 98,893.6 $\pm$ 14,834	(5D)	(5D)
II (chloroquine)	116,207.75 $\pm$ 87,038.8	37,736 $\pm$ 336	237.1 $\pm$ 145.96	0	0
III (lamivudine)	184,111.24 $\pm$ 30,237	52,890.5 $\pm$ 8,113.5	31,172.22 $\pm$ 7,914.2	(3D) 33,082.71 $\pm$ 0	(5D)
IV (zidovudine)	62,132.87 $\pm$ 22,816.1	74,705.25 $\pm$ 16,397.7	30,512.54 $\pm$ 21,880.2	621 $\pm$ 0 (NS)	0
V (nevirapine)	162,629.96 $\pm$ 59,030.6	55,244.23 $\pm$ 9,003.39	75,267.7 $\pm$ 770.6	(3D) 86,584.5 $\pm$ 7,966.5	(5D)
VI (stavudine)	103,219.46 $\pm$ 32,230.5 (NS)	65,378.35 $\pm$ 15,985.0	27,718.4 $\pm$ 15,221.8	(4D) 169,655.2 $\pm$ 5,333.5	(5D)
VII (LZN)	31,583.53 $\pm$ 6,361.67 (NS)	29,717.01 $\pm$ 1,157.11	30,671.3 $\pm$ 22,276	(3D) 0 (NS)	(3D)0
VIII (LSN)	29,784.95 $\pm$ 8,507.11	29,117.48 $\pm$ 9,705.83 (NS)	47,030.7 $\pm$ 10,091.0	(5D)	(5D)

*Data are expressed as mean $\pm$ SEM for five animals per group.

D = no. animals that died; 0 = no malaria parasites; NS = not significantly varied from chloroquine, $P > 0.05$ (parasite densities of other groups varied significantly, $P < 0.05$); LZN = lamivudine/zidovudine/nevirapine; LSN, lamivudine/stavudine/nevirapine.

TABLE 2
Effect of prophylactic lamivudine, zidovudine, nevirapine, and stavudine on *Plasmodium berghei* infection in adult albino mice

Drug group	Parasite densities/ μ L of blood sampled on different days*				
	Day 4	Day 6	Day 8	Day 20	Day 26
I (distilled water)	67,794.5 $\pm$ 9,616.0	71,866.25 $\pm$ 7,418.26	(1D) 98,893.6 $\pm$ 14,834.04	(5D)	(5D)
II (chloroquine)	116,207.75 $\pm$ 87,038.8	37,736 $\pm$ 336	237.1 $\pm$ 145.96	0	0
IX (lamivudine)	123,966.02 $\pm$ 44,605.6 (NS)	168,014.26 $\pm$ 76,389.2	44,345.59 $\pm$ 10,339.5	(2D) 136,073.42 $\pm$ 52,290.4	(5D)
X (zidovudine)	62,474.85 $\pm$ 14,639.1	65,792.43 $\pm$ 31,011.2	27,429.47 $\pm$ 9,282.2	2,601.33 $\pm$ 0 (NS)	0
XI (nevirapine)	367,352.95 $\pm$ 74,933.2	(1D) 185,726.01 $\pm$ 48,968.1	(1D) 94,641.61 $\pm$ 28,210	(2D) 87,571.44 $\pm$ 5,050.31	(5D)
XII (stavudine)	0 (NS)	11,477.99 $\pm$ 2,786.24	64,541.07 $\pm$ 10,544.7	(5D)	(5D)
XIII (LZN)	41,138.1 $\pm$ 3,528.03	34,554.95 $\pm$ 11,111.6 (NS)	15,913.84 $\pm$ 929.44 (NS)	(1D) 0 (NS)	(1D) 0
XIV (LSN)	67,987.69 $\pm$ 6,210.04	28,428.75 $\pm$ 828.8 (NS)	38,105.73 $\pm$ 1,719.6	(5D)	(5D)

*Data are expressed as mean $\pm$ SEM for five animals per group.

D = no. animals that died; 0 = no malaria parasites; NS = not significantly varied from chloroquine, $P > 0.05$ (parasite densities of other groups varied significantly, $P < 0.05$); LZN = lamivudine/zidovudine/nevirapine; LSN, lamivudine/stavudine/nevirapine.

the rate of parasite uptake may have been distorted, which would have confounded the initial picture of parasitemia and parasite elimination within the first few days.

Results were subjected to statistical analysis by using the Student's *t*-test. Significance was set at a probability level of 0.05. Results are given as mean $\pm$ SEM.

RESULTS

Animals receiving curative lamivudine had an initial decrease in mean malaria parasite density from 184,111.24 $\pm$ 30,237 parasites/ μ L to 31,172.22 $\pm$ 7,914.2 parasites/ μ L. This decrease was followed by an upward trend between days 12 and 18 and another decrease by day 20 (Table 1). Mice receiving curative regimens of zidovudine had consistently decreasing mean malaria parasitemia from day 8, when it was 30,512.54 $\pm$ 21,880.2 parasites/ μ L and total malaria parasite clearance by day 26. Mice receiving curative nevirapine had periods of decrease and increase of mean malaria parasite density values. Mice receiving stavudine had an initial decrease of mean parasite densities followed by an increase: the initial density was 103,219.46 $\pm$ 32,230.5 parasites/ μ L and the final density was 169,655.2 $\pm$ 5,333.5 parasites/ μ L. Mice receiving curative LZN had a decrease in mean malaria parasite density from 31,583.53 $\pm$ 6,361.6 parasites/ μ L on day 4 to 7,667.84 $\pm$ 1,541.24 parasites/ μ L on day 12 and complete

malaria parasite clearance by day 18. Mice receiving curative LSN had increasing mean parasite densities up to day 12, and all animals died by day 18.

Animals receiving prophylactic lamivudine had an initial increase followed by a decrease and eventually another period of increase in mean density from day 12. Animals started to die from day 20, when the mean $\pm$ SEM density was 136,073.42 $\pm$ 52,290.4 parasites/ μ L (Table 2). The mean $\pm$ SEM malaria parasite density of mice receiving prophylactic zidovudine decreased from 62,474.85 $\pm$ 14,639.1 parasites/ μ L on day 4 to 2,601.33 $\pm$ 0 parasites/ μ L on day 20, and total malaria parasite clearance occurred by day 26. Mice receiving prophylactic nevirapine had a remarkable period of decrease in mean parasite density from an initial value of 367,352.95 $\pm$ 74,933.2 parasites/ μ L to 59,195.58 $\pm$ 2,052.29 parasites/ μ L on day 18. Subsequently, there was an increase in the mean $\pm$ SEM parasite density by day 20 to 87,571.44 $\pm$ 5,050.31 parasites/ μ L, after which all animals died. Mice receiving prophylactic stavudine had an initial increase followed by a constant level of mean malaria parasite density.

Mice receiving prophylactic LZN had a consistent decrease in mean $\pm$ SEM malaria parasite density from 41,138.1 $\pm$ 3,528.03 parasites/ μ L until all parasites were eventually eliminated by day 20. Mice receiving prophylactic LSN had an initial decrease, followed by a period of increase in mean $\pm$ SEM malaria parasite density. Mice receiving prophylactic

FIGURE 1. Four-day suppressive test of the effect of antiretroviral drugs on *Plasmodium berghei*. Chloroquine (CQ) vs. lamivudine; CQ vs. zidovudine; CQ vs. nevirapine; CQ vs. lamivudine/zidovudine/nevirapine (LZN). P values were < 0.05 for all comparisons.

TABLE 3

Average malaria parasite (*Plasmodium berghei*) clearance/day for lamivudine, zidovudine, and nevirapine in adult albino mice

Regimen	Drug	Mean $\pm$ SEM clearance/day/ μ L*	P
Curative	Zidovudine	2,389.73 $\pm$ 877.4	< 0.05
	LZN	1,754.6 $\pm$ 353.4	
Prophylactic	Zidovudine	2,402.86 $\pm$ 562.1	> 0.05
	LZN	2,181.48 $\pm$ 197.4	

*Five animals per group.

LZN = lamivudine/zidovudine/nevirapine.

zidovudine and LZN had relatively lower mean malaria parasite densities during the experiment.

The Peters four-day suppressive test showed percent suppression for lamivudine, zidovudine, nevirapine, chloroquine, and LZN to be 67.0%, 69.0%, 76.9%, 100%, and 82.2%, respectively (Figure 1). Suppressive values for antiretroviral drugs were significantly different from that for chloroquine ($P < 0.05$).

Mean $\pm$ SEM malaria parasite clearance/day for mice receiving curative zidovudine, curative LZN, prophylactic zidovudine, and prophylactic LZN were 2,389.73 $\pm$ 877.4 parasites/ μ L, 1,754.6 $\pm$ 353.4 parasites/ μ L, 2,402.86 $\pm$ 562.1 parasites/ μ L, and 2,181.48 $\pm$ 197.4 parasites/ μ L, respectively (Table 3). Zidovudine administered as curative and prophylactic regimens resulted in greater malaria parasite clearance/day compared with LZN. Mean malaria parasite clearance/day for the curative regimen of zidovudine was significantly higher than that for LZN ($P < 0.05$). However, no significant difference was noted in groups that had prophylactic courses of these drugs ($P > 0.05$).

The PRR of curative regimens were 3.48, 0.83, 2.94, and 1.58 for lamivudine, zidovudine, nevirapine, and stavudine, respectively. Curative regimens of LZN and LSN were 1.06 and 1.02, respectively. Also, the group receiving distilled water had a PRR of 0.94 and the group receiving chloroquine had a PRR of 3.08. There was no significant difference between these values ($P > 0.05$).

DISCUSSION

This study showed that the antiretroviral drugs lamivudine, zidovudine, and nevirapine have antimalarial properties and average % suppressive values of 67.0%, 69.0% and 76.9%, respectively. The triple combination of LZN had an average % suppressive value of 82.2%. Although these values were significantly different from that for chloroquine ($P < 0.05$), the suppressive activity of these drugs is nonetheless important because it has the potential of reducing parasite biomass.

Two scenarios can be found among HIV-infected patients in malaria-endemic regions with regard to exposure to malaria infection. In the first scenario, malaria parasites may be present in the patient's blood sample before antiretroviral treatment is started. Most persons are constantly bitten by mosquitoes and are expected to have malaria parasitemia, although they eventually show development of immunity against malaria. Therefore, clinical manifestation of malaria will not occur as frequently as expected and malaria fever episodes will not be severe. This finding is the classical case, particularly if such a person lives in the region. In the second scenario, a person infected with HIV who has had a course of effective antimalaria treatment and was subsequently treated

with antiretroviral drugs subsequently gets infected with malaria. These two case scenarios were simulated in our study, which established the antimalaria properties of curative and prophylactic regimens of zidovudine and LZN, which in both cases eradicated *P. berghei* in mice. Noteworthy is the fact that parasite densities of mice receiving zidovudine and LZN were not significantly different from those receiving chloroquine toward the end of the study. Thus, the pharmacologic action of curative and prophylactic regimens of zidovudine and LZN suggests that HIV-infected patients who are receiving these drugs in malaria-endemic regions such as in sub-Saharan Africa may be cured of malaria parasitemia, although clinical research is needed to validate this theory. If such a theory is true, it is expected that eradication of malaria parasitemia in these patients will occur whether there had been parasitemia before beginning treatment with antiretroviral drugs or malaria infection occurred after HIV-infected patients received antiretroviral drugs.

First-line antiretroviral therapy in HIV-infected patients in sub-Saharan Africa is LZN; most HIV-infected patients in the region receive this therapy. Thus, it is expected that there will be simultaneous control of malaria among these patients and by extension a reduction in the prevalence of malaria parasitemia even among non-HIV-infected persons against the backdrop that malaria transmission from HIV-infected patients receiving these antiretroviral drugs will be reduced. Therefore, this study has resolved the question raised in a previous report about the effect of LZN on malaria infection.³⁵

Zidovudine has been administered as single drug therapy for preventing mother-to-child transmission of HIV-1 in pregnant women who did not yet need to receive antiretroviral therapy.³⁵ It is expected that such use of zidovudine would obviously eradicate malaria parasitemia in the mother and may also prevent malaria transmission to the child.³⁶

Zidovudine administered alone resulted in a higher average malaria parasite clearance/day than LZN in the mice groups that had either curative or prophylactic regimens. The difference between zidovudine clearance rate and that of LZN was significant among the curative groups, and there was no significant difference between the prophylactic groups (2,389.73 $\pm$ 877.4 parasites/ μ L and 1,754.6 $\pm$ 353.4 parasites/ μ L; $P < 0.05$, and 2,402.86 $\pm$ 562.1 parasites/ μ L and 2,181.48 $\pm$ 197.4 parasites/ μ L; $P > 0.05$, respectively). Thus, zidovudine appears to have antimalaria properties as illustrated by the comparison of malaria clearance rates of zidovudine and LZN, and also because lamivudine, stavudine, nevirapine, or LSN did not eradicate malaria parasitemia (parasitemia increased with these drugs). It should be noted that eradication of malaria parasites by zidovudine and LZN occurred over a chronic period of exposure to these drugs. This finding does not necessarily indicate that these drugs are potential antimalarial drugs because antimalarial drugs used for curative purposes in humans need to eradicate parasites rapidly.³⁴ However, the models we have simulated are still relevant as far as malaria/HIV interaction is concerned. Drugs such as antiretroviral drugs, which have the potential of decreasing malaria parasite biomass in HIV-infected patients are relevant for HIV therapy in malaria-endemic regions and the choice of such antiretroviral drugs should be encouraged in the absence of any contraindication.

Malaria enhances multiplication of HIV,^{2,26-29} this multiplication may lead to HIV disease progression and increased

HIV infectivity. This study suggests that administration of zidovudine and LZN to HIV-infected patients may reverse this clinical deterioration and infectivity expected to be induced by malaria. Use of zidovudine or LZN in the management of HIV infection in sub-Saharan Africa may be an advantage because these antiretroviral therapies are capable of eradicating malaria parasites. Therefore, we expect that HIV multiplication will be reduced. The administration of these drugs to HIV-infected patients in malarious regions may modify the epidemiology of malaria and therefore the HIV pandemic.

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Authors' addresses: Akinwumi Akinyede, Alade Akintonwa, Olufunsho Awodele, Sunday Olayemi, and Ibrahim Oreagba, Department of Pharmacology, College of Medicine, University of Lagos, Lagos, Nigeria, E-mails: a_akinyede@yahoo.co.uk, toxicologyresearch@yahoo.com, awodeleo@yahoo.com, olayemiso@yahoo.com, and oreagbai@yahoo.com. Oluwagbemiga Aina and Samuel Akindele, Department of Biochemistry and Nutrition, Nigeria Institute of Medical Research, Yaba, Lagos, Nigeria, E-mails: gbengaaina2003@yahoo.com and samuelakindele@yahoo.com. Charles Okany, Department of Hematology and Blood Transfusion, College of Medicine, University of Lagos, Lagos, Nigeria, E-mail: charlesokany@yahoo.com.

Reprint requests: Akinwumi Akinyede, Department of Pharmacology, College of Medicine, University of Lagos, PMB 12003, Lagos, Nigeria, E-mail: a_akinyede@yahoo.co.uk.

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