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Interaction between chloroquine sulphate and aqueous extract of *Azadirachta indica* A. Juss (*Meliaceae*) in rabbits

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This study was carried out to investigate the effect of concurrent oral administration of aqueous leaf extract of *Azadirachta indica* (*Meliaceae*) on the pharmacokinetic properties of chloroquine sulphate in experimental rabbits. The results indicated that concurrent administration of both resulted in a significant decrease in serum concentration, slower absorption and elimination as well as longer half-life of chloroquine sulphate. The highest relative decrease of 78.0% was recorded 4 hours after concurrent administration, while the smallest decrease (64.6%) occurred 24 hours after concurrent administration. Significant reductions were also noted in some pharmacokinetic parameters of chloroquine and included the area under the curve (71.9%), maximum serum concentration (69.8%), absorption rate constant (37.3%), elimination rate constant (53.9%), clearance rate (76.5%) and volume of distribution (47.2%). However, there was a pronounced increase in the half-life of the drug (125.7%).

Keywords: chloroquine sulphate, *Azadirachta indica* (*Meliaceae*), pharmacokinetic interaction

Correct use of an effective antimalarial drug will not only shorten the duration of malaria but will also reduce the incidence of complications and the risk of death (1). Development of new drugs is not keeping pace with the challenging trends of malaria in Africa and some developing countries; in addition, few effective, affordable alternatives to chloroquine are available (2). Chloroquine is a weak base and an alkaloid blood schizontocide (3). The use of chloroquine as a single first line drug treatment is now increasingly limited following the evolution of chloroquine-resistant *Plasmodium falciparum*. Nevertheless, the low cost and easy availability makes chloroquine the drug of choice for most patients in Africa.

The use of herbal preparations in the treatment of malaria is popular in many parts of Africa and Asia where malaria infestation is endemic. *Azadiracta indica* A. Juss (*Meliaceae*), in many countries referred to as the »neem tree« or »Dogon yaro«, has been extensively reported as being effective in the treatment of malaria caused by various strains of

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plasmodium, even those resistant to traditional antimalarial drugs (4–7). Various phytochemical constituents have been isolated from the plant and demonstrated to possess antimalarial properties (8). Some of these include flavonoid quercetin and rutin (8) and limonoid gedunin, which have been reported to be as effective as quinine in malaria infected cell cultures (7, 9). The antimalarial activity of *A. indica* was found to be due to its ability to induce oxidant stress in erythrocytes during malaria treatment; this redox perturbation leads to the death of the parasite in erythrocytes (4). It produces significant changes in the biochemistry of the liver, like increasing the microsomal protein content and enhancing the aniline hydroxylase activity.

The established use of chloroquine as well as of *A. indica* extracts as effective antimalarials offers the possibility of concurrent administration of both agents. This study was designed to investigate the influence of concurrent administration of aqueous extract of *A. indica* and chloroquine on the pharmacokinetic properties of the latter in experimental rabbits.

EXPERIMENTAL

Materials and instruments

The following materials were used in the study: chloroquine sulphate (May and Baker, Products, UK), Nivaquine Fort®, a brand of chloroquine sulphate (May and Baker Pharmaceutical PLC, Nigeria), fresh leaves of *A. indica* (collected from neem trees at the campus of the University of Nigeria, Nsukka), five healthy adult male rabbits weighing between 1.05 to 1.41 kg (bought from a local market in Nsukka, Nigeria).

Digital pH meter (P107, Consort, Belgium), ultraviolet-visible spectrophotometer (SP 8–100, Pye Unicam, UK), centrifuge (Beckman GS-15, UK), were used.

Methods

Botanical identification of fresh leaves of *A. indica* was confirmed by H. M. Ekekwe of the Department of Botany, University of Nigeria, Nsukka. The extract was prepared as follows: fresh leaves of *A. indica* (580 g) were thoroughly mashed in distilled water (2 L). The decoction was filtered using a clean sieve cloth. Its concentration was determined by evaporating the extract to dryness. Freshly prepared extracts were used. Concentration of the aqueous extract was found to be 15.25 mg mL^{-1} dry extract and pH was 5.2, indicating a weakly acidic extract. The extractive yield (expressed as dry mass of the extract relative to the mass weight of the leaves) was determined to be 4.2%. The extract was concentrated so as to prepare 100 mg mL^{-1} dry extract solution.

The five healthy adult male rabbits were kept in the experimental Animal House Unit of the Department of Pharmacology and Toxicology, University of Nigeria (Nsukka, Nigeria) for seven days with free access to food and water before the commencement of the experiment. The animals were starved for 12 h before each phase of the experiment and fed 1 h after administration of the drugs. Ethical standards of the University were adhered to in the course of the study.

Determination of chloroquine sulphate in the rabbits' sera was done in two phases. In both phases, Nivaquine Fort® (a brand of chloroquine sulphate) was suspended in 3% Tween 85 to give 100 mg mL^{-1} suspension. The animals were given between 0.16 and 0.21 mL of the above chloroquine sulphate suspension, depending on their body mass. Both chloroquine sulphate and the extract were administered to the animals orally through a cannula inserted through the oral cavity. Phase one of the experiment commenced by administration of 15 mg kg^{-1} of chloroquine sulphate to each rabbit orally. This dosing was based on the 15 mg kg^{-1} loading dose of chloroquine sulphate in humans (10). Blood samples (1 mL) were aseptically withdrawn from the marginal ear vein of the rabbits at 1, 2, 4, and 24 h intervals after drug administraton. Serum samples were obtained after centrifugation at 3000 rpm for 10 min and stored in a refrigerator (10–15 °C) until analyzed.

After blood withdrawal in the first phase, the rabbits were allowed a one-week drug 'wash-out' period before the commencement of the second phase and during that period they had free access to food and water. The second phase started on the 8th day by administration of 15 mg kg^{-1} of chloroquine sulphate orally to each rabbit and 100 mg kg^{-1} of aqueous extract of *A. indica* was immediately administered orally to each rabbit. The animals received between 1.1 and 1.4 mL of the aqueous extract relative to their body mass. Blood samples were collected at similar time intervals as in phase one and analyzed as described above. For each animal, the serum concentration of chloroquine sulphate was compared for the drug administered alone with that administered concomitantly with the aqueous extract.

Analysis of chloroquine sulphate in the serum was carried out at λ_{max} of 328 nm. A drug-free serum was used as the control. The respective concentrations were obtained from the Beer-Lambert plot, which was obtained using standard solutions of pure chloroquine sulphate (160, 80, 40, 20 and 10 μg mL^{-1}).

The area under the curve (*AUC*), absorption rate constant (k_a), elimination rate constant (k_e), half life ($t_{½}$), peak serum concentration (c_{max}), volume of distribution (V_d) and serum clearance rate (*cl*) were determined as described before (11).

The results were expressed as mean ± standard error of the mean. Significance of the difference between the control and test values was evaluated using Student's *t*-test. This was done using the computer programme 'Statistical Package for Social Sciences (SPSS)', version 7.5. $p < 0.05$ was taken as the significance level.

RESULTS AND DISCUSSION

Concurrent administration of the aqueous extract and chloroquine sulphate significantly decreased ($p < 0.05$) the serum concentration of the latter agent (see Table I). The decrease in serum concentration of chloroquine recorded 1, 2, 4 and 24 hours after drug administration was 69.8%, 74.5%, 78.0% and 64.6%, respectively.

The peak concentration of chloroquine was 1 h in both groups and an almost constant concentration was maintained for 4 h up to 24 h regardless of extract administration. This may be the result of the long half-life of chloroquine (Table II); consequently, the serum level was maintained at a relatively constant concentration for a long time.

Table I: Serum concentration of chloroquine sulphate administered alone and given concurrently with aqueous extract of A. indica

Time (h)	Serum concentration (μg mL^{-1})	
	Chloroquine alone[a]	Chloroquine + *A. indica* extract[a]
1	2.8 ± 0.6[b]	0.8 ± 0.3[b]
2	1.8 ± 0.6[c]	0.4 ± 0.1[c]
4	1.2 ± 0.2[d]	0.3 ± 0.04[d]
24	1.1 ± 0.4[e]	0.4 ± 0.07[e]

[a] Mean ± SEM, $n = 5$.
[b–e] Statistically significant difference ($p < 0.05$) between the group with *A. indica* extract and the group without extract.

This finding is consistent with previous reports indicating that chloroquine has a long half-life and that appreciable concentration of the drug was detected in the plasma several days after cessation of the therapy (12, 13). The long half-life of the drug was been attributed to extensive tissue binding, particularly in liver, spleen, kidney, lung, melanin containing tissues, and, to a lesser extent, brain and spinal cord.

Apart from the time for maximum serum concentration (t_{max}), concurrent administration of chloroquine sulphate and aqueous extract of *A. indica* significantly changed all the pharmacokinetic parameters of chloroquine sulphate (Table II). The concurrent administration resulted in a significant decrease ($p < 0.05$) of *AUC*, c_{max}, k_a, k_{el}, c_l and V_d (Table II). The aqueous extract of *A. indica* has been reported to significantly increase microsomal proteins (4), and chloroquine has been documented to have mean values for

Table II. The pharmacokinetic parameters of chloroquine sulphate alone and with aqueous extract of A. indica

Pharmacokinetic parameter	Chloroquine alone[a]	Chloroquine + *A. indica* extract[a]	Change (%)/ comment
AUC (μg mL^{-1} h^{-1})	28.9 ± 1.0[b]	8.1 ± 0.8[b]	–71.9/decrease
c_{max} (μg mL^{-1})	2.8 ± 0.4[c]	0.8 ± 0.3[c]	–69.8/decrease
t_{max} (h)	1.0	1.0	no change
$t_{1/2}$ (h)	26.65 ± 5.23[d]	60.16 ± 6.13[d]	125.7/increase
Clearance rate (h^{-1})	5.88 ± 1.01[e]	1.38 ± 0.46[e]	–76.5/decrease
k_a (h^{-1})	0.67 ± 0.18[f]	0.42 ± 0.12[f]	–37.3/decrease
k_{el} (h^{-1})	0.026 ± 0.0098[g]	0.012 ± 0.004[g]	–53.9/decrease
V_d (L kg^{-1})	225.99 ± 12.68[h]	119.39 ± 10.15[h]	–47.2/decrease

[a] Mean ± SEM, $n = 5$.
[b–h] Statistically significant difference ($p < 0.05$) between the group with *A. indica* extract and the group without extract.

protein binding ranging between 58–64% (14). Since protein bound drugs are not free as to be detected in the serum or plasma and are not metabolized (11), the observed decreases in elimination rate constant, clearance rate and serum concentration of chloroquine may be attributed to enhancement of chloroquine binding to microsomal protein, leading to a decrease in free drug in the serum and thereby a decrease in the above parameters. Such binding may also explain the observed increase in half-life because protein binding helps extend the half-life of a drug by gradual release of the drug over a long period.

From the foregoing, it is evident that concomitant administration of chloroquine sulphate and aqueous extract of neem impaired the bioavailability of chloroquine, and thereby it may adversely affect the therapeutic efficacy of chloroquine. However, as various classes of chemotherapeutic agents exert different actions on microorganisms, one drug has the potential to either enhance or inhibit the effect of another (15). In addition, the strategy of combining drugs with different modes of action and mechanisms of resistance, a standard approach in the treatment of tuberculosis and HIV infection is advocated as a way to improve antimalarial therapeutic effectiveness and delay the emergence of drug resistance (2). Decoctions of the leaves and bark of *A. indica* have been claimed to be efficacious in the treatment of malaria caused by various strains of plasmodium, even where orthodox drugs have been found ineffective (4). We do not know whether aqueous extract of *A. indica* enhances or antagonizes the antimalarial effectiveness of chloroquine in a clinical setting. However, because of the inherent antimalarial potency of *A. indica*, it is possible that the aqueous extract may be complementing the antimalarial effect of chloroquine. In this regard, it is premature, from the findings of this study, to predict an antagonistic therapeutic outcome of the concurrent administration of the tested agents.

CONCLUSIONS

Concurrent administration of chloroquine sulphate and aqueous extract of *A. indica* impaired the bioavailability and decreased most of the pharmacokinetic parameters of chloroquine. However, due to the claimed antimalarial potency of the aqueous extract and the fact that various chemotherapeutic agents exert different actions on microorganisms, and as such have the potential to either enhance or inhibit the effect of the other agent, at this stage of investigation we may not predict with certainty, the clinical effect of such concurrent administration. Further studies are required to investigate the outcome in animal models of malaria.

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S A Ž E T A K

Interakcija između klorokin sulfata i vodenog ekstrakta biljke Azadirachta *indica* A. Juss (*Meliaceae*) u zečeva

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U radu je ispitivan učinak istodobne peroralne primjene vodenog ekstrakta listova biljke *Azadirachta indica* (*Meliaceae*) na farmakokinetičke parametre klorokin sulfata na zečevima kao eksperimentalnim životinjama. Rezultati ukazuju da istodobna primjena tih dvaju ljekova dovodi do značajnog smanjenja koncentracije u serumu, sporije apsorpcije i eliminacije, te produljenja vremena polueliminacije klorokin sulfata. Najveće relativno smanjenje od 78,0% zapaženo je 4 sata, a najmanje (64,6%) 24 sata nakon istodobne primjene. Zabilježeno je i značajno smanjenje nekih farmakokinetičkih parametara klorokina: površine ispod krivulje (71,9%), maksimalne koncentracije u serumu (69,8%), brzine apsorpcije (37,3%), brzine eliminacije (53,9%), klirensa (76,5%) i volumena distribucije (47,2%). Međutim, vrijeme polueliminacije značajno je produljeno (125,7%).

Ključne riječi: klorokin sulfat, *Azadirachta indica* (*Meliaceae*), farmakokinetska interakcija

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