Suppression of the chloroquine resistance of *Plasmodium berghei* by treatment of infected mice with a microsomal monoxygenase inhibitor*

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Administration of a combination of chloroquine and the copper-lysine complex, copper(lysine), an inhibitor of microsomal monoxygenases, considerably decreased the parasitaemia level of mice infected with a chloroquine-resistant strain of *Plasmodium berghei*. When given separately, chloroquine and the complex had no antimalarial effect. Use of a combination of monoxygenase inhibitors and chloroquine therefore appears to be a promising addendum to the chemotherapy of malaria caused by chloroquine-resistant parasites.

One of the major causes of the recent resurgence of malaria in tropical countries has been the spread of strains of *Plasmodium* spp. that are resistant to chloroquine and other antimalarial drugs (1). It has been suggested that the increased prevalence of the chloroquine-resistant strains has arisen because of the spread of mutants that are naturally selected for enhanced enzymic inactivation of chloroquine due to the intense therapeutic use of the drug.⁷ Many toxic xenobiots and drugs are inactivated and eliminated from eukaryotic cells by a set of microsomal monoxygenases, of which cytochrome P-450 plays a key role (2). Chloroquine is also deactivated by these enzymes in a process that involves de-ethylation and hydroxylation (3). Malarial parasites also possess microsomal monoxygenases, and the activity of these enzymes in chloroquine-resistant strains of the rodent malarial parasite *Plasmodium berghei* is higher than in the chloroquine-sensitive strain (4). Compounds that inhibit these monoxygenases have been proposed as promising agents for overcoming the chloroquine resistance of the malarial parasites, and, of those inhibitors reported, the copper-lysine complex, copper(lysine), has the greatest inhibition effect *in vitro* on the activity of *P. berghei* microsomal monoxygenases (4). Here we report the results of a study of the *in vivo* suppression of the chloroquine resistance of *P. berghei* in infected mice.

**MATERIALS AND METHODS**

The experiments were performed on random-bred mice that were infected with the syringe-passaged laboratory strain ChIR LNK-65 of *P. berghei* that had acquired resistance to chloroquine. The strain was produced artificially from strain LNK-65.⁸ Preliminary experiments indicated that the ChIR LNK-65 strain is 8–10 times more resistant to chloroquine than the natural drug-sensitive strain of *P. berghei*. In order to maintain the acquired level of chloroquine resistance, mice were given a single oral dose of the drug before each successive syringe

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⁷ Kindly supplied by Professor W. Peters, Liverpool School of Tropical Medicine, Liverpool, England.
passage at the maximum tolerated level (500 mg per kg body weight).

Chloroquine diphosphate containing 62% chloroquine base was used. The copper(lysine) complex was synthesized as already described (5). Mice were administered a 500 mg/kg or 250 mg/kg single oral dose of chloroquine. Based on the results of previous studies (4), a dose of 20 mg/kg of the complex given intraperitoneally in saline was used to inhibit microsomal monoxygenase activity.

Infection of mice and evaluation of parasitaemia

White random-bred mice weighing 12–14 g were injected intraperitoneally with 0.1 ml of infected blood containing about $1.5 \times 10^7$ of P. berghei parasites. Altogether five groups of mice, each consisting of 8–10 males with developed parasitaemia, were used in the study.

Chloroquine was administered orally in a single dose of 250 mg/kg to mice in group I, while a dose of 500 mg/kg was given to those in group II. Mice in group III were treated with a combination of chloroquine (250 mg/kg orally) and the copper complex (20 mg/kg intraperitoneally). Group IV received only an intraperitoneal injection of 20 mg/kg of the copper complex, while mice in group V received no treatment and served as controls. The drugs were given 2 days after the mice in groups I-IV had been infected.

Infection of mice with the ChLR LNK-65 strain of P. berghei is associated with frequent spontaneous remission of symptoms, clearance of parasitaemia, and eventual cure. Evaluation of the effectiveness of chloroquine, either alone or in combination with copper (lysine), was therefore based on the course of the infection. Daily counts were made of parasitized erythrocytes from the first day of treatment until remission of the disease, i.e., until parasitaemia had cleared. The level of parasitaemia was evaluated using the scoring system described by Rabinovich & Moshkovsky (6).

RESULTS

The course of the infection in the controls and treated mice is shown in Fig. 1. In the controls, parasitaemia developed progressively to a high level during the first 5 days of the infection and this level was maintained over the following 11 days. Half of the mice died within 16 days of infection; however, the rest underwent spontaneous remission leading to clearance of parasitaemia. Up to day 17 of the study the course of infection of controls was the same as that of mice treated only with the copper complex. However, the latter mice showed no remission and all died between days 13 and 24. In the first 5 days after its administration, chloroquine inhibited slightly the development of parasitaemia among mice of group I, but during the subsequent 8 days parasitaemia developed at the same rate as in the control mice of group V. There followed a spontaneous remission of parasitaemia and cure for 50% of the mice in group I, while the rest died.

The number of parasitized erythrocytes in the controls was compared with the number in mice that had been given the chloroquine–copper(lysine)$_2$ combination (group III). On average, the number of parasitized erythrocytes decreased by 2.6 units the day following treatment and continued to fall over the subsequent 2–3 days (Fig. 1). On day 6 the number of parasitized erythrocytes in group III was smaller by 4.8 units than that in the controls, by 4.7 units than that in group I, and by 3.3 units than that in group II. Subsequently, the number of parasitized erythrocytes increased in group III, presumably because the single dose of chloroquine-copper(lysine)$_2$ administered 3 days after the infection had ceased to be effective.

Although parasitaemia increased among the mice of group III, its level up to the 11th day of the
infection was lower than that in the controls and in those groups that received either chloroquine or the copper complex alone (Fig. 1).

DISCUSSION

The results we have obtained indicate that inhibitors of enzymes which inactivate chloroquine and facilitate its removal from *P. berghei* cells can restore the antimalarial activity of the drug. Furthermore, the experiments demonstrated that copper(lysine), on its own exhibits no antimalarial activity.

It cannot be excluded that the activity of chloroquine may be enhanced also by the inhibitory effect of the copper complex on liver microsomal monooxygenases, thereby prolonging the circulation of higher concentrations of the drug. This inhibition may be a factor that contributes to the action of the copper complex on chloroquine activity, since an increase in the dose of chloroquine from 250 mg/kg to 500 mg/kg body weight did not appreciably promote its antimalarial action.

The results we have described, together with those of previous studies, offer a novel approach, i.e., the use of chloroquine synergists, to overcoming the drug resistance of malarial parasites. It is hoped that chloroquine can be used therapeutically also with other compounds that potentiate its antimalarial effects.

Enzymes that metabolize xenobiotics might also be responsible for the development of resistance of *Plasmodium* spp. to other drugs also. Thus, use of inhibitors of these enzymes to circumvent any acquired resistance has potential as an effective antimalarial strategy.

RÉSUMÉ

SUPPRESSION DE LA CHLOROQUINORÉSISTANCE DE *PLASMODIUM BERGHEI* CHEZ LA SOURIS AU MOYEN D’UN INHIBITEUR DES MONOXYGÉNASES MICROSUMALES

Des études antérieures ont montré que les mono-oxynénasases étaient plus actives chez des souches de *Plasmodium berghei* chloroquinorésistantes que chez des souches pharmacosensibles du parasite. Le complexe cuivre-lysine (Cu(lysine)₃) est in vitro un inhibiteur efficace des monoxygenasases microsomales et il est proposé d'utiliser cette action pour venir à bout des plasmodies chloroquinorésistantes.

Les résultats dont nous fassions état montrent que l'association de chloroquine et de cuivre(lysine) réduit sensiblement la parasitémie chez des souris infestées par des souches de *Plasmodium berghei* chloroquinorésistantes. Administrés séparément, la chloroquine et le complexe cuivre-lysine n'ont aucun effet antipaludique. Par conséquent, l'utilisation d'inhibiteurs des monoxygenasases, associés à la chloroquine, est prometteuse en chimiothérapie du paludisme à *Plasmodium* chloroquinorésistant.

REFERENCES