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Potentiation of the antimalarial action of chloroquine in rodent malaria by drugs known to reduce cellular glutathione levels

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Abstract

Ferriprotoporphyrin IX (FP) is released inside the food vacuole of the malaria parasite during the digestion of host cell hemoglobin. FP is detoxified by its biomineralization to hemozoin. This process is effectively inhibited by 4-aminoquinolines. As a result FP accumulates in the membrane fraction and associates with enzymes of infected cells in parallel with parasite killing. Free FP is degraded by reduced glutathione (GSH). This degradation is inhibited by chloroquine (CQ) and amodiaquine (AQ) but not by quinine (Q) or mefloquine (MQ). Increased GSH levels in *Plasmodium falciparum*-infected cells confer resistance to CQ and vice versa, and sensitize CQ-resistant *Plasmodium berghei* by inhibiting the synthesis of glutathione. Some drugs are known to reduce GSH in body tissues when used in excess, either due to their pro-oxidant activity or their ability to form conjugates with GSH. We show that acetaminophen, indomethacin and disulfiram were able to potentiate the antimalarial action of sub-curative doses of CQ and AQ in *P. berghei*- or *Plasmodium vinckei petteri*-infected mice, but not that of Q and MQ. In contrast, *N*-acetyl-cysteine which is expected to increase the cellular levels of GSH, antagonized the action of CQ. Although these results imply that alteration in GSH are involved, measurement of total glutathione either in uninfected or *P. berghei*-infected mice, treated with these drugs did not reveal major changes. In conclusion, experimental evidences provided in this study suggest that some off the counter drugs can be used in combination with some antimalarials to which the parasite has become resistant.

Keywords: Malaria; Chloroquine; Drug potentiation; *Plasmodium berghei*; *Plasmodium vinckei petteri*; Acetaminophen; Indomethacin

1. Introduction

We have recently resolved the involvement of glutathione in the antimalarial action of CQ. This can be summarized as follows [1]: ferriptoroporphyrin IX (FP) is released during the digestion of host cell hemoglobin within the food vacuole of the parasite. FP is biocrystallized into hemozoin in a process that is effectively inhibited by 4-aminoquinoline antimalarial drugs [2]. In the presence of these drugs, free FP exits the food vacuole into the parasite's cytosol where it can be degraded by glutathione (GSH). FP degradation is effectively inhibited by CQ and AQ, but not by Q or MQ [3]. Undegraded FP accumulates in the membrane fraction of infected cells [4], and binds to some parasite proteins [5], in parallel with parasite killing. As the inhibition of GSH-mediated degradation of FP by CQ and AQ is competitive [3], it explains the ability to increase resistance to CQ by increasing the levels of GSH in *Plasmodium falciparum*-infected erythrocytes and vice versa [1], and to render *Plasmodium berghei* that were selected for CQ resistance *in vivo*, sensitive to the drug in presence of an inhibitor of glutathione synthesis [6]. Interestingly, the generation of CQ resistance in *P. berghei* resulted in a marked increase in [GSH] and glutathione-*S*-transferase (Gst) activity, and remarkably, a sharp reduction of hemozoin levels [7]. These results have led the authors to suggest that GSH (probably provided by the host during ingestion of the cytosol of the infected cell) inhibits the biocrystallization of FP and destroys it. The correlation between [GSH] and CQ resistance is supported by the

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Abbreviations: APAP, acetaminophen; AQ, amodiaquine; CQ, chloroquine; DDC, *N*,*N*-diethyldithiocarbamate; DIS, disulfiram; FP, ferriprotoporphyrin IX; GSH, reduced glutathione; Gst, GSH-*S*-transferase; INDO, indomethacin; MQ, mefloquine; NAC, *N*-acetyl-cysteine; NAPQI, *N*-acetyl-*p*-benzoquinone imine; PMNs, polymorphonuclear leukocytes; Q, quinine.

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recently reported higher expression of γ-glutamyl-cysteine synthetase, the first enzyme in the glutathione biosynthetic pathway [8]. This deliberation does not mean that altered levels of GSH is the sole mechanism of resistance to CQ. The parasite obviously possesses other means, such as mutations in the Pgh1 drug pump [9] and in the membrane protein pfcrt [10]. From the above description it becomes apparent that the combination of a drug that can reduce [GSH] *in vivo* could potentiate the antimalarial action of CQ and thus re-establish it as a first line therapy for malaria, irrespective of the location of GSH action. In cancer cells, drug resistance can also be associated with cellular GSH. This involves the formation of GSH-drug adducts that is mediated by GSH-*S*-transferase (Gst) and the up-regulation of the multiresistance protein (MRP) that pumps GSH-drug adducts from treated cells, thereby reducing intracellular drug concentration [11]. A gene coding MRP has been predicted recently in the genome of *P. falciparum* [12]. Beyond the action of the pump, the mere uptake of nucleophiles would result in depletion of GSH since the parasite expresses Gst [13]. In addition, as the level of GSH in the host compartment of infected cells is below normal to start with [14], depletion of its GSH should compromise the antioxidant defense and result in membrane alterations that predisposes them to phagocytosis [15,16].

Acetaminophen (*N*-acetyl-*p*-aminophenol; APAP) is one of the most extensively used analgesics/antipyretics worldwide. It is usually considered as a safe drug but overdose or idiopathic reaction can cause major morbidity and mortality in both humans and experimental animals [17–19]. APAP is primarily detoxified in the liver by glucoronidation and sulfation, but when these systems are saturated by overdoses, APAP is transformed to the reactive metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) by CYPE1 and CYP1A2 that are isoforms of cytochrome P450. NAPQI arylates proteins [20] and is detoxified by GSH [21]. Thus, treatment of mice with a sub-toxic overdose of APAP resulted in massive depletion of GSH and pretreatment with glutathione isopropyl ester was found to protect against APAP-induced liver damage [22]. The effects of APAP in renal tubules is potentiated by enzymatic metabolism of APAP by the liver to NAPQI, possibly involving oxidation and GSH conjugation [23] and the NAPQI-derived thioether conjugates are present in bile, urine, and feces [24]. Thus, NAPQI produced in the liver could essentially diffuse out of this organ and reach other tissues, including the blood where it could deplete GSH in erythrocytes.

Indomethacin (INDO), one of the most effective non-steroidal anti-inflammatory drugs, is widely used clinically to treat inflammatory diseases, including rheumatoid arthritis and gout. The anti-inflammatory action of this drug is due to inhibiting prostaglandin synthesis by preventing cyclooxygenase activity of prostaglandin H synthase [25,26]. INDO has been shown to activate polymorphonuclear leukocytes (PMNs) in peripheral blood of rats that were administered the drug at 30 mg/kg per o.s., thereby instigating these cells to enhanced release of oxygen radicals [27]. Increased oxidative stress induced by PMNs interfere with the growth of *P. falciparum* [28,29] be it caused by the presence of specific opsonizing anti-*P. falciparum* antibodies retrieved from malaria patients [30] or by induction of TNF-α production [31]. As such, it could potentially reduce glutathione levels inside the parasite.

Disulfiram (DIS) is a dithiocarbamate (DC) used as an adjunct in the treatment of chronic alcoholism. It inhibits aldehyde dehydrogenase that is responsible for the oxidation of acetaldehyde produced by alcohol dehydrogenase after the intake of alcohol. The accumulation of acetaldehyde causes the acetaldehyde syndrome whose adverse symptoms fortify the desire to stop drinking [32]. In addition to binding metals, the free thiol groups of DCs can also react with sulfhydryl groups on other molecules. DCs have thus been reported to inhibit enzymes by covalent interaction to free protein thiols. In the presence of GSH, DIS is reduced to *N*,*N*-diethyldithiocarbamate (DDC) nonenzymatically, with a stoichiometric relationship of 2:1 [33]. In erythrocytes, DDC reacts with the super-oxo-ferriheme complex of oxyhemoglobin to generate hydrogen peroxide and DIS and the cyclic conversion of oxyhemoglobin to methemoglobin and DDC to DIS results in the net oxidation of GSH [34]. DIS does not deplete total glutathione but significantly reduces the GSH/GSSG ratio [35,36]. DDC has an antimalarial activity *in vitro* that is potentiated by sub-toxic concentrations of copper. It was therefore suggested that DDC forms a complex with copper, either intracellularly or extracellularly, which is toxic to malarial parasites [37].

In this study, the ability of APAP, INDO and DIS to enhance the antimalarial action of CQ and AQ has been tested in two mouse malaria models *in vivo*. It was found that such enhancement does indeed occur as evidenced by the reduction of parasitemia and the improvement of the survival of infected mice. As expected, such enhancement has not been found with Q or MQ.

2. Materials and methods

2.1. Testing of combinations of 4-aminoquinolines and APAP or INDO in the P. berghei mouse model

Groups of five to seven Swiss mice each were inoculated by intraperitoneal injection of 10^6 to 10^7 erythrocytes infected with *P. berghei* NK65. The 4-day test of Peters and Robinson [38] was used to assess sub-curative doses of various 4-aminoquinolines (CQ, AQ, MQ or Q) in order to achieve less than full drug effect. The effect of various doses of APAP and INDO and their combinations with the aminoquinoline antimalarials were similarly tested. The doses used are shown in Section 3. Treatment started 2 hr post-infection and repeated on days 1, 2 and 3, one group

receiving placebo of drug carrier. Parasitemia was assessed on day 4 by microscopic inspections of Giemsa-stained thin blood smears.

2.2. Testing of combinations of 4-aminoquinolines and DIS or NAC in the P. vinckei mouse model

ICR mice were inoculated by blood passage, injecting intraperitoneally 10^6 to 10^7 cells infected with *Plasmodium vinckei petteri*. Drug treatment (i.p. injection) started either shortly (~2 hr) after infection or when infected cells could be detected in Giemsa-stained thin blood smears made from tail blood, usually 3 days after infection. Groups of six to seven infected mice each were either left as controls with no drug, or treated for 4 days with chloroquine sulfate dissolved in saline, or mefloquine sulfate dissolved in DMSO, or DIS dissolved in DMSO, or *N*-acetyl-cysteine (NAC) dissolved in saline, or combinations of chloroquine or mefloquine with either DIS or NAC. The amounts of chloroquine and mefloquine were chosen to be sub-curative. The dose of NAC was double that used to treat rats for acetaminophen intoxication [39]. The doses used are shown in Section 3. The toxicity of DIS to mice has been first tested, and the highest sub-toxic dose has been selected. Drug effects were checked both by monitoring the evolution of parasitemia and by the survival of infected mice.

2.3. Measurement of total glutathione in drug-treated mice

Stock solutions of APAP (20 mg/mL), DIS (4.8 mg/mL) and INDO (20 mg/mL) were prepared in DMSO and NAC (60 mg/mL) was prepared in PBS. Concentrations of drugs were adjusted so that each mouse was given 0.1 mL of drug solution. For control, mice were injected with 0.1 mL of either PBS or DMSO. Uninfected mice or mice infected with *P. berghei* (15–30% parasitemia), three mice in each group, were injected intraperitoneally to reach drug concentrations shown in the Section 3. At times 0, 1, 2, 4 hr (as well as 24 hr for uninfected mice) each mouse was bled from the tail. Five drops of blood were mixed with 5 mL of PBS containing 20 μL heparin. The tubes were kept on the ice until processed. Blood samples were washed once with PBS, the white layer of lymphocytes was removed and the cells were counted. To each pellet of cells was added 0.5 mL of freshly prepared 1% (w/v) sulfosalicylic and the lysates were placed on ice (for at least 15 min). The lysates were centrifuged 5 min at 10,000 *g* and total glutathione in the supernatant was measured as previously described [6] with minor modifications: 100 μL of 0.1 N HCl were mixed with DTNB-containing buffer (110 mM Na_2HPO_4, 40 mM NaH_2PO_4, 0.04% (w/v) bovine serum albumin, 15 mM EDTA and 0.3 mM DTNB). To this mixture were added 100 μL of sample and 200 μL reagent mix (50 mM HCl–imidazole (pH 7.2), 0.02% bovine serum albumin, 2 mM NADPH and 1 unit/mL of Type IV yeast glutathione reductase). A calibration curve was prepared from GSH (0.3–50 μM) freshly prepared in 1% sulfosalicylic acid. Optical density at 412 nm was read for 10 min at room temperature. Glutathione concentrations per 10^{10} cells were calculated.

2.4. Statistical analysis

The significance of treatment effect has been evaluated for particular groups of data (as indicated in the legends to the respective figures) using the Wilcoxon–Mann–Whitney test.

3. Results

3.1. Interactions between APAP or INDO and 4-aminoquinolines in P. berghei-infected mice

Results depicted in Fig. 1 show that APAP (at concentrations that do not have any effect on parasite growth, see Fig. 2) potentiates the antimalarial effect of CQ given at sub-curative dose. However, a minimal dose of 1.5 mg/kg CQ is needed to elicit the effect. INDO itself has a minimal and insignificant inhibitory effect at 50 mg/kg, and it increases the antimalarial effect even with a CQ dose of 0.75 mg/kg and a dose of INDO of 20 mg/kg. This potentiation is more pronounced at a dose of 50 mg/kg INDO and 1.5 mg/kg CQ.

Essentially the same effects are seen with AQ, a congener of CQ (Fig. 2). APAP at 200 mg/kg and INDO at 100 mg/kg do not affect the progress of infection. AQ at 2 mg/kg is only partially inhibiting the evolution of parasitemia, and its

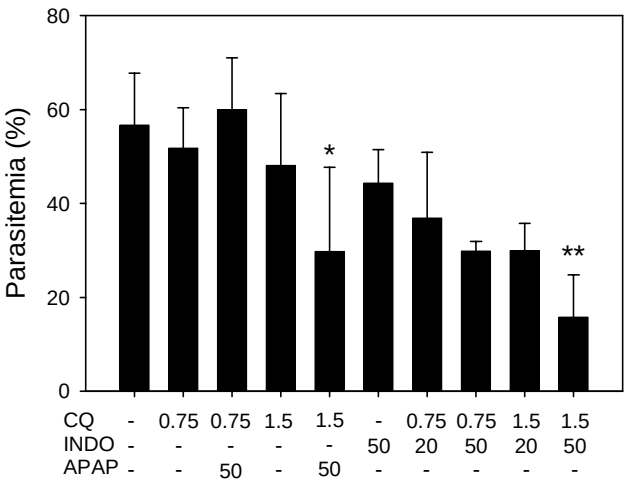

Fig. 1. Antimalarial effects of CQ, INDO, APAP and their combinations. Mice were infected with *P. berghei* and drug treatment started ~2 hr after infection for 4 consecutive days. Drug doses are indicated in mg/kg. Parasitemias were evaluated on day 5 post-infection. Results show means ± SD. $^{*}P$-value comparing CQ 1.5 and CQ 1.5 + APAP 50 (12 control and 12 treated mice) = 0.0039; $^{**}P$-value comparing CQ 1.5 and INDO 50 + CQ 1.5 (16 control and 10 treated mice) <0.00001.

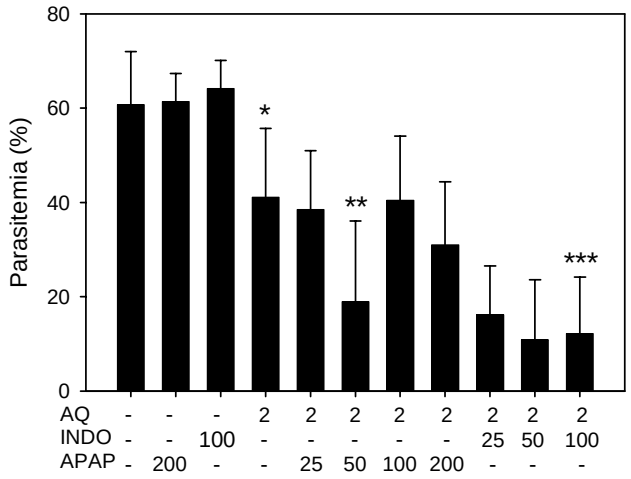

Fig. 2. Antimalarial effects of AQ, INDO, APAP and their combinations. Mice were infected with *P. berghei* and drug treatment started ~2 hr after infection for 4 consecutive days. Drug doses are indicated in mg/kg. Parasitemias were evaluated on day 5 post-infection. Results show means ± SD. $^{*}P$-value comparing control and AQ2 (36 mice in each group) <0.00001; $^{**}P$-value comparing AQ2 and AQ2 + APAP 50 (36 mice in AQ group and 30 mice in AQ + APAP group) <0.00001; $^{***}P$-value comparing AQ2 and AQ2 + INDO 100 (36 mice in AQ group and 12 mice in AQ2 + INDO 100 group) <0.00001.

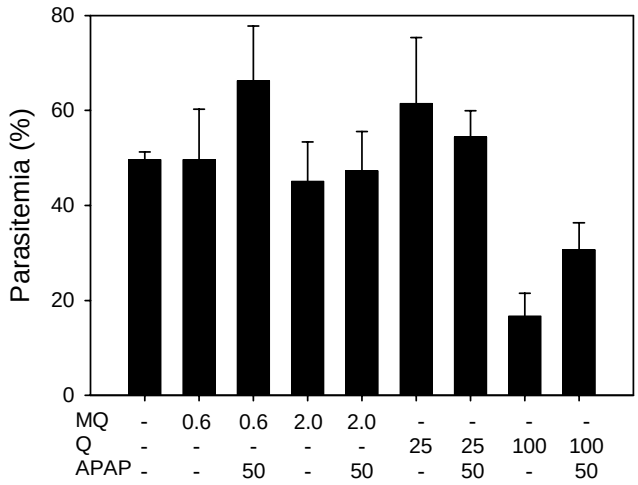

Fig. 3. Antimalarial effects of Q and MQ and their combination with APAP. Mice were infected with *P. berghei* and drug treatment started ~2 hr after infection for 4 consecutive days. Drug doses are indicated in mg/kg. Parasitemias were evaluated on day 5 post-infection. Results show means of six mice ± SD.

action is enhanced by administering doses of APAP considerably lower than 200 mg/kg, although this effect is not dose-dependent. This is also the case for the potentiation of AQ activity by doses of INDO lower than 100 mg/kg. For both drugs there is a decrease in the enhancement of AQ action at the highest concentration used. We tentatively assign this decreased enhancement to plausible excessive decrease in GSH that may preclude the maintenance of essential SH moieties of the vacuolar H^{+}-pump [40]. This activity is needed for the accumulation of AQ inside the parasite's food vacuole.

In contrast to the observed synergism between CQ or AQ and the supposed depletors of GSH, the combinations of APAP with MQ or Q, has no effect on parasitemia. As can be seen from Fig. 3, sub-therapeutic levels of MQ or Q were identified and the addition of APAP had no effect on the evolution of parasitemia and some times (MQ/APAP 0.6/50, Q/APAP 100/50) there was even an increase in the parasitemia. The combination of MQ or Q with INDO, were found to be toxic to the mice (only one to three out of five mice survived the treatment).

3.2. Effects of DIS and NAC on the antimalarial effects of 4-aminoquinolines in P. vinckei petteri-infected mice

Results shown in Fig. 4 were obtained with mice that were treated immediately after infection of the mice. Notice for this figure and the following ones that parasitemia relates only to the surviving mice. In untreated control mice parasitemia evolved to high levels (Fig. 4A) and resulted in death, starting on day 7 post-infection (Fig. 4B). CQ (1.5 mg/kg) reduced parasitemia and mortality only to a small extent as intended. DIS (12 mg/kg) had no effect either on the evolution of parasitemia or on mortality. However, the combination of CQ and DIS was protective: parasitemia was reduced to almost nil and survival of infected mice was considerably increased. The parasitemic pattern suggests that the reduction of the parasite load allows the surviving mice to mount immune response and self-cure. The same experiment has been repeated but in this case, drug treatment started only on day 3 post-infection when parasitemia in the infected mice became evident (0.1–1.0%). The same effects on the evolution of parasitemia were obtained in principle (Fig. 5A). Comparison of the two experimental protocols, shows that mice survival in the presence of DIS was slightly reduced but was considerably increased with the CQ–DIS combination (Fig. 5B). It is impossible to decide if these differences are due to batch variability or to treatment protocol.

Unlike the synergistic interaction between CQ and DIS, that between MQ and DIS was without effect on the evolution of parasitemia (Fig. 6A). In this experiment MQ reduced parasitemia and allowed self-resolution of the infection, whereas DIS had no effect. The combination of MQ and DIS was not different from MQ alone. Mice survival followed parasitemia except for the drug combination that afforded less protection to the mice compared with MQ alone (data not shown). This experiment has been repeated with halved MQ concentration (1.0 mg/kg). In this case, neither MQ alone nor MQ + DIS had any effect on the parasitemia (data not shown), but MQ improved survival and here again the combination was the less protective (Fig. 6B).

NAC is expected to increase the levels of glutathione in cells [41] and therefore, according to the rationale of this investigation, should antagonize the curative effect of CQ. The assay of NAC was warranted as a positive control.

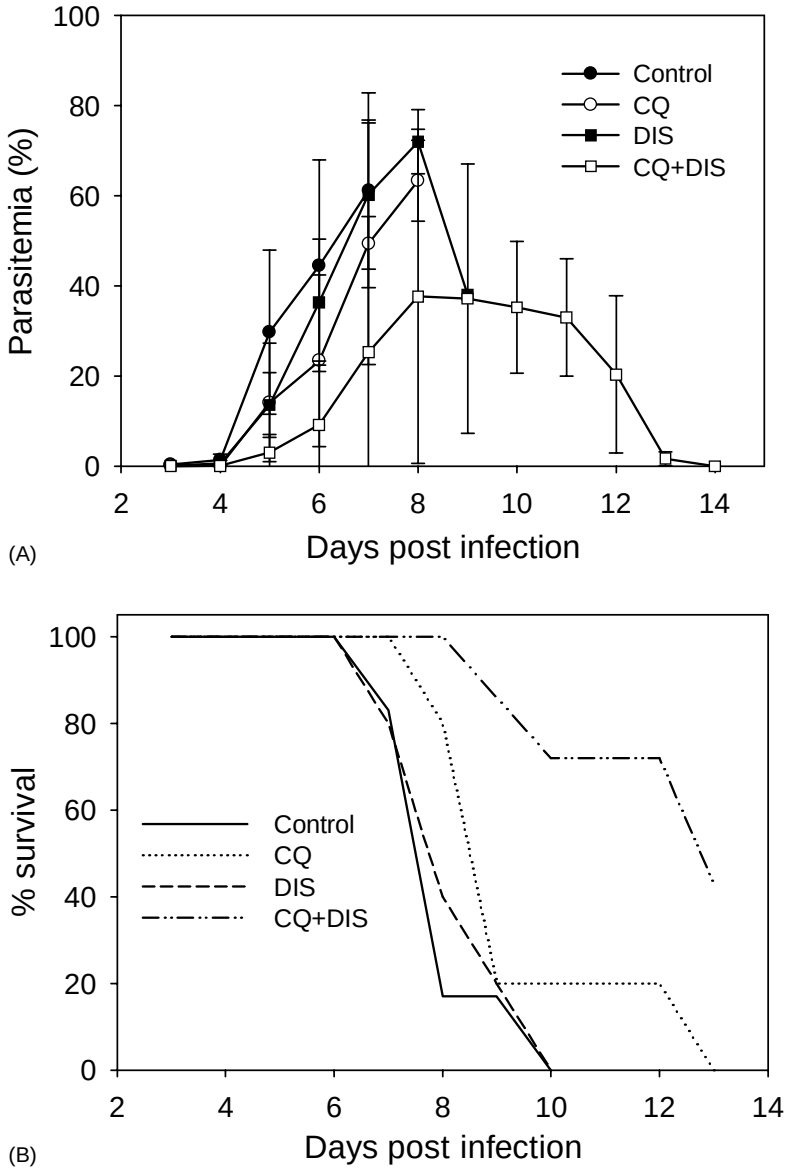

Fig. 4. Antimalarial effects of CQ, DIS and their combination. Mice were infected with *P. vinckei petteri* and drug treatment started ~2 hr after infection for 4 consecutive days. CQ was administered at 1.5 mg/kg and DIS at 12 mg/kg. Parasitemia was checked daily (A) and the survival of mice (B) was followed until day 13 post-infection. Results show means of six mice ± SD. Statistical analysis of significance has been performed comparing CQ- and CQ + DIS-treated animals. *P*-value for days 5, 6 and 7 was <0.05 and for day 8 was <0.5. Beyond 9 days statistical analysis is meaningless because of high mortality of CQ-treated mice (see part B).

Results shown in Fig. 7 indicate that NAC exacerbated the evolution of parasitemia (Fig. 7A) and decreased the survival of mice (Fig. 7B). These results may suggest that the antioxidant defense of infected cells is deficient and NAC could upgrade it and thus enhance parasite proliferation. Combination of CQ and NAC was able to reduce the effect of CQ, as expected. But this effect could also be interpreted as the reversal of the effect of NAC.

3.3. Effects of APAP, INDO, DIS and NAC on the levels of glutathione in uninfected and P. berghei-infected mice

In order to verify if the various alleged modulators had an effect on the levels of glutathione, total glutathione has been measured in the blood of drug-treated uninfected and in *P. berghei*-infected mice (15–30% parasitemia). Since it was desirable to test drug effects over time, the amounts of blood withdrawn had to be limited. For this reason only total glutathione has been measured rather than differentially GSH and GSSG. Since there were differences between the levels of glutathione among the mice, drug effects were calculated with respect to the level at time zero that was measured prior to treatment. As can be seen in Fig. 8A, treatment of healthy mice with different drugs barely affected the levels of erythrocyte GSH. Values obtained at 24 hr post-treatment were low even in controls, suggesting that stress due to blood sampling may be accountable. In infected mice, DMSO cause an increase

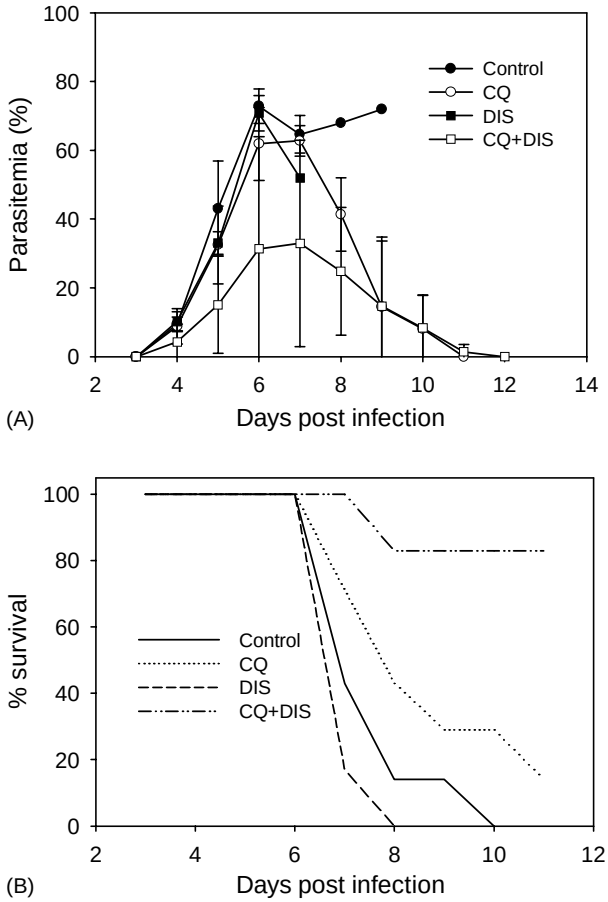

Fig. 5. Antimalarial effects of CQ, DIS and their combination. Mice were infected with *P. vinckei petteri* and when infection became evident in blood smears on day 3 post-infection, drug treatment started and followed for 4 consecutive days. CQ was administered at 1.5 mg/kg and DIS at 12 mg/kg. Parasitemia was checked daily (A) and the survival of mice (B) was followed until day 13 post-infection. Results show means of six mice ± SD. Statistical analysis of significance has been performed comparing CQ- and CQ + DIS-treated animals. *P*-value for days 4 and 5 was <0.05, for day 6 <0.08, for day 7 <0.2 and for day 8 <0.25. Beyond 9 days statistical analysis is meaningless because of high mortality of CQ-treated mice (see part B).

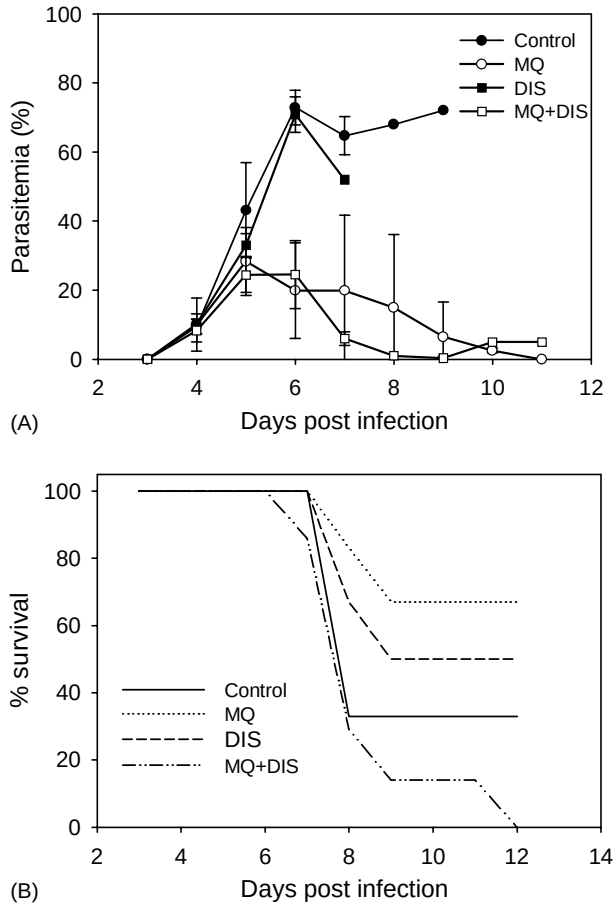

Fig. 6. Antimalarial effects of MQ, DIS and their combination. Mice were infected with *P. vinckei petteri* and when infection became evident in blood smears on day 3 post-infection, drug treatment started and followed for 4 consecutive days. MQ was administered at 2.0 mg/kg and DIS at 12 mg/kg. Parasitemia was checked daily (A) and the survival of mice (B) was followed until day 13 post-infection. Results show means of six mice ± SD.

in glutathione and this could have affected the expected effects of DIS and INDO that were given dissolved in DMSO. In infected mice (Fig. 8B), INDO produced and immediate and sustained reduction in GSH, while APAP produced a delayed transient effect, probably due to the need to produce NAPQI and allow it to distribute out of the liver. The effect of other drugs was minimal.

4. Discussion

Both in cultures of *P. falciparum* [1] and in *P. berghei*-infected mice [6,7], the reduction of GSH leads to sensitization of the parasites to CQ. This perception of the involvement of GSH in the antimalarial mode of action of CQ has directed us to test the interaction between drugs that are expected to deplete cells from GSH and 4-aminoquinolines. Three compounds were chosen to test their ability to modulate the antimalarial action of the 4-aminoquinolines: APAP was expected to interfere directly with GSH, whereas INDO and DIS were expected to affect the GSH/GSSG ratio. Experiments were done with two different malaria mouse models to ascertain that the effects are not species-specific. All three drugs, as well as NAC, did not alter the levels of total glutathione in the erythrocytes of uninfected mice. In infected mice INDO caused a rapid and sustained reduction in total glutathione and APAP generated a delayed transient reduction. These marginal effects are most probably due to their use at subtoxic doses. It is also possible that drug treatment has also changed the GSH/GSSG ratio, but this could not be tested for technical reasons. Increased levels of GSSG could have enhanced the efflux of the disulfide. However, all three drugs substantially potentiated the antimalarial action of CQ and AQ as assessed by the levels of parasitemia (and in the case of DIS, also in the prolongation of survival of infected mice), but not those of Q or MQ. Since that antimalarial action of CQ and AQ involves GSH but that of Q or MQ does not, these results suggest that minor

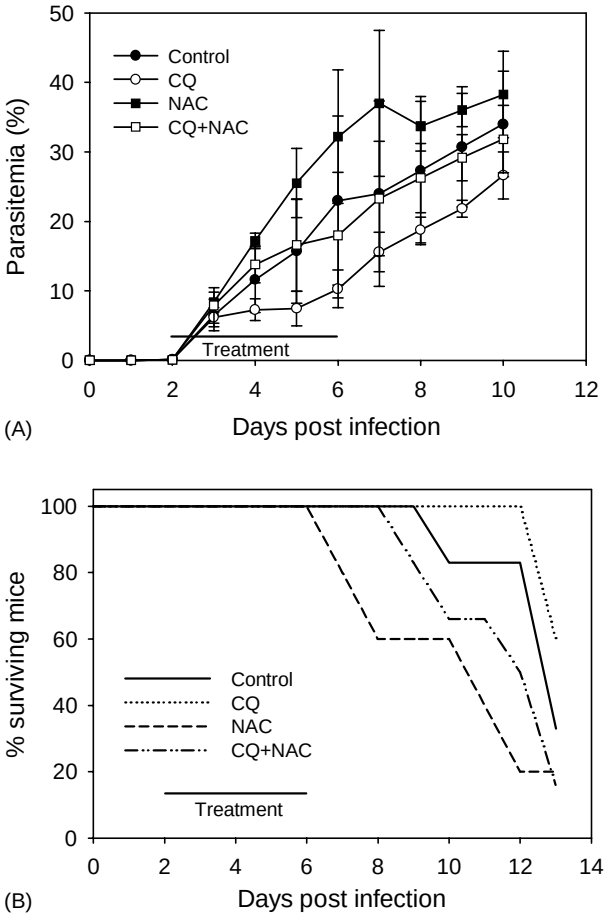

Fig. 7. Antimalarial effects of CQ, NAC and their combination. Mice were infected with *P. vinckei petteri* and when infection became evident in blood smears on day 3 post-infection, drug treatment started and followed for 4 consecutive days. CQ was administered at 1.5 mg/kg and NAC at 300 mg/kg. Parasitemia was checked daily (A) and the survival of mice (B) was followed until day 13 post-infection. Results show means of six mice ± SD.

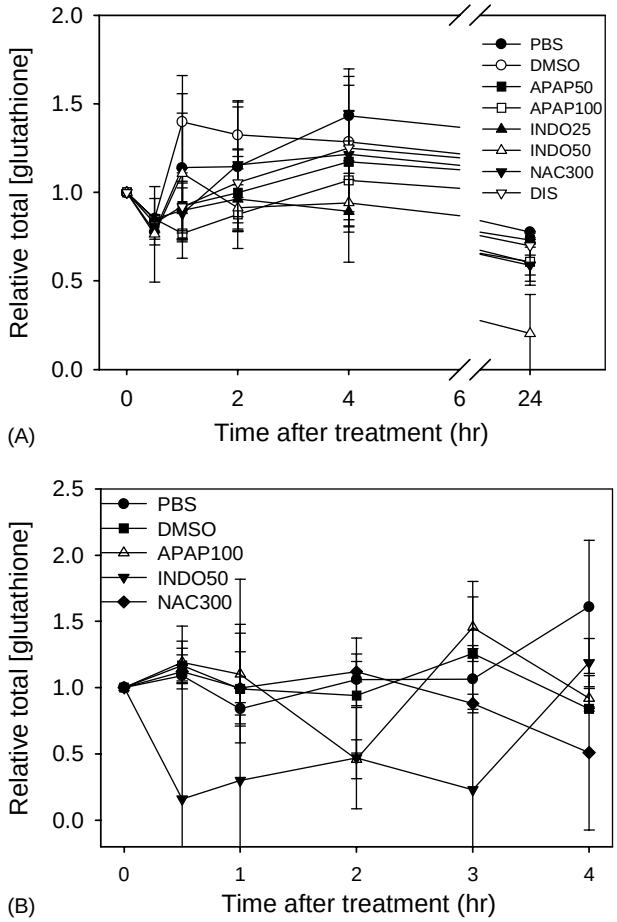

Fig. 8. Effect of drugs on the level of glutathione in erythrocytes of uninfected mice and mice infected with *P. berghei*. (A) Healthy mice in groups of three were injected with different drugs. (B) Mice were infected with *P. berghei* and when the parasitemia reached 15–30% groups of three were injected with different drugs. At the indicated times, blood samples were withdrawn from the tail and total glutathione concentration has been determined, as described in Section 2. Values at each time were normalized to the value at time zero for each mouse in order to account for individual variations. Results are the means ± SD of two independent experiments. Results of INDO and APAP were compared to DMSO control because drugs were dissolved in this solvent. For INDO: at 30 min and 1 hr $P < 0.3$, for 2 hr <0.06 and for 3 hr <0.15; for APAP at 2 hr <0.3.

changes in GSH levels were at the base of the observed differential interactions. Concordant with these results were the observed antagonistic effects between CQ and NAC, since NAC is expected to increase the level of GSH and thus increase the resistance to CQ [1]. Metabolic activation of APAP leads to increased synthesis of nitric oxide and superoxide and thus to peroxynitrite [18]. In this way as well it could have assisted in limiting parasitemia because parasites are sensitive to NO and oxidative radicals [42].

APAP has been tested for its antipyretic activity in malaria, and found to be efficient in reducing fever [43]. Whereas in combination with Q, it prolonged parasite clearance time [44], a greater degree of parasite clearance was achieved sooner in combination with CQ [45]. In most of these investigations, relatively low concentrations of APAP (up to 50 mg/kg per day at 6-hr intervals) were administered. The discrepancies between the effects of APAP on treatments with CQ and Q agree with the differential interactions of CQ or AQ and Q or MQ with GSH in their antimalarial action [3,4]. They were also seen in this study, whereas the antimalarial activities CQ and its congener AQ were potentiated by APAP, those of Q and MQ were not.

Several studies were performed to test the metabolization of APAP in *Plasmodium*-infected rats. It has been suggested that infection may compromise important pathways of drug detoxification [46–48]. Other studies provided contrasting results [49]. It was found however in human patients of *falciparum* malaria that the metabolism and disposition of APAP are not affected by clinical malaria [50,51]. It may be worthwhile to test the combination of APAP and CQ or MQ using somewhat higher concentrations of the analgesic.

The synergistic combination of INDO and CQ or AQ that was found in this study can be evaluated from an additional angle. INDO inhibits prostaglandins synthesis

[52–54]. Although the production of prostaglandins by *P. falciparum* is not affected by INDO [55], INDO enhanced lymphoproliferative responses to malaria antigens, indicating that prostaglandins may have a generalized immunosuppressive role in malaria-infected individuals [56]. Furthermore, it has been recently shown that elevated prostaglandin E2 in healthy malaria-exposed children may protect against malaria [57]. Thus, the effects of INDO observed in this study may be also related to enhanced immune response.

In conclusion, the results of this study demonstrate that some off-the-counter drugs can be used to potentiate the antimalarial action of CQ and AQ *in vivo*. It may mean that combination of CQ or AQ with these drugs can serve to treat patients of *falciparum* malaria that are infected with CQ-resistant strains. Can the present results pave the way for clinical trials in human malaria patients? The maximum tolerated dose of APAP has recently been reported to be 15 g/m^2 [58]. This is equivalent to ~400 mg/kg, significantly higher than the 50 mg/kg used in this study. The daily permissible intake of INDO in humans is 300 mg or ~4 mg/kg. This would not suffice for eliciting the potentiation effect of this drug. For DIS, 1.5 g per day (20 mg/kg) has no ill effect even when taken for weeks. This is above the dose of 12 mg/kg used in this study. The data for INDO and DIS were found in http://healthanswers.telstra.com/drugdata/. This discussion disregards the possible differences in pharmacokinetics between mouse and man, but it certainly indicates the feasibility of this approach for the treatment of human patients infected with CQ-resistant parasites.

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