EFFECT OF ANTIMALARIAL DRUGS AND OF CLINDAMYCIN ON ERYTHROCYTE METABOLISM.
A REVIEW.

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The resistance of Plasmodium falciparum to many drugs and the reduced number of new antimalarial drugs accepted for clinical use, have led to a tendency to treat malaria patients attacked by resistance strains with a combination of drugs18, 19, 20, 21, 25, 27, 39, 40, 54. For this reason, it is necessary to learn about the interaction among the available drugs to which the parasite is sensitive.

The classic antimalarial drugs (chloroquine, primaquine, amodiaquine, proguanil, sulfones, sulfonamides, pyrimethamine and mefloquine) are characterized by having a benzene ring in their molecular structure16 and therefore are oxidants capable inducing of the superoxide radicals (O_2^-) production26. These radicals are very reactive and capable of producing oxidative stress12-33 inside the erythrocyte in addition to contributing to parasite death26, 29, 37. It should be pointed out that this is not the main pharmacological mechanism in the fight against plasmodia, though these parasites are particulary sensitive to O_2^- radicals due to their high polyunsaturated lipid content and weak antioxidant defense system22, 38.

In the red blood cells of normal individuals, superoxide dismutase transforms O_2^- to H_2O_2,33, which is later transformed to H_2O and O_2 under the action of catalase and glutathione peroxidase6, 10, 33. Glutathione peroxidase needs appropriate levels of reduced glutathione23. The latter, after being oxidized, is reduced by glutathione reductase, which in turn depends on flavin adenine dinucleotide (FAD) and NADPH8, 9, 10, 11, 12. FAD is a phosphorylated riboflavin derivative and NADPH is generated by the pentose pathway, glucose-6-phosphate dehydrogenase (G6PD) being the modulating enzyme of the entire system8, 9, 10, 11, 12. The diagram below is a summary of the main effects of antimalarial drugs on erythrocyte metabolism8, 10, 12, 33.

Individuals with inborn or acquired defects in these metabolic pathways such as G6PD-, glutathione reductase-, glutathione peroxidase and riboflavin-deficient subjects may suffer hemolysis when treated with these drugs6, 8, 9, 10, 11, 12, 38, 46. This is due to the fact that the oxidative stress on the erythrocyte ultimately leads to the formation of methemoglobin, oxidation of SH groups and peroxidation of red blood cell membrane lipids. These alterations produce Heinz bodies, leading to hemolysis and consequent anemia6, 8, 10, 26, 46.

Among genetic defects, G6PD deficiency is quite frequent in populations living for a long time in regions where malaria is endemic6, 38, 46.

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This deficiency is intimately related to the hemolytic anemia caused by antimalarial drugs\(^3\), \(^4\), \(^6\). In a study of the prevalence of G6PD-deficient individuals in a population from an endemic zone of malaria, BARRAVIERA et al.\(^8\) detected 5% prevalence in the Amazonian population of Humaitá (AM). This value was close to that reported for the population of the Brazilian Northeast\(^1\) and higher than that detected among Indians (0%)\(^4\). MEIRA et al.\(^4\), in a study of the frequency of histocompatibility antigens, pointed out the higher rate of undetermined antigens (blancks) among Amazonians. JOBIM et al.\(^3\) obtained similar results when studying histocompatibility antigens among Tukuna Indians. These authors\(^3\), \(^4\) attributed the elevated rate of undetermined antigens to the phenomenon of homozygosis, frequently occurring among native populations.

**Effect of antimalarial drugs on erythrocyte metabolism**\(^9\), \(^10\), \(^12\)

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\text{Reduced glutathione (GSH)} \rightarrow \text{glutathione reductase} \rightarrow \text{glutathione peroxidase} \rightarrow \text{O}_{2}^{-} + \text{H}_{2}\text{O}\]

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\text{glutathione peroxidase} \rightarrow \text{NADPH} \rightarrow \text{glucose} \rightarrow \text{glucose 6-phosphate dehydrogenase} \rightarrow \text{6-phosphogluconate} \rightarrow \text{NADP}^{+}
\]

Colauto et al.\(^2\) pointed out the homogeneous behavior of blood group systems in the Amazonian population of Humaitá, with a prevalence of group 0, positive Rh. The same authors\(^3\) detected a similar frequency of hemoglobinopathy S among the inhabitants of villages located along the basin of the Madeira river in the township of Humaitá when compared with the population of the Northeast.

It should be emphasized that the Amazonian population of Humaitá may be a representative sample of large part of the Amazonians living in the Amazon region. This because, according to Azevedo\(^3\), the Amazon region was strongly affected by the Northeastern element in its colonization. However, the natives also participated in the formation of these populations\(^4\). Ultimately, all indications are to the effect that Indians and inhabitants of the Northeastern hinterland played an important role in the formation of the Amazonian population. The G6PD deficiency and hemoglobinopathy S genes may have been brought by the Northeasterners and the increased prevalence of these defects may be related to the selective pressure exerted by malaria\(^4\).

This is relevant, since these genetic defects carried by the genes of Negroes\(^8\), \(^10\), \(^4\) on the one hand are related to resistance to malaria\(^3\) and on the other may play a fundamental role in the hemolytic anemia triggered by drugs\(^3\), \(^8\), \(^9\).

Another interesting, probably acquired phenomenon observed among Amazonians is the fact that these individuals have decreased glutathione reductase activity\(^6\). Hereditary deficiency of this enzyme is very rare, with few cases reported thus far\(^3\), \(^3\). However, this deficiency in general is related to riboflavin deficiency\(^6\), \(^8\), \(^9\), \(^10\). Barraviera et al.\(^9\) detected decreased glutathio-
ne reductase activity in approximately 80% of the individuals studied. Among the factors contributing to this phenomenon are the feeding habits of the Amazonians, who subsist on manioc flour and fish and consume reduced amounts of vegetables, legumes, fruits or eggs, which are foods rich in riboflavin. Furthermore, this population shows a high rate of infestation by the parasite *Ascaris lumbricoides*, a helminth that depletes albumin, vitamin C and riboflavin.

On the other hand, studies on animals fed riboflavin-free diets and inoculated with *Plasmodium* have shown attenuated infection and low parasitemia. BATES et al. and THURNHAM et al. found riboflavin deficiency among children living in regions where malaria is endemic. THURNHAM related these findings to physiological adaptation that results in decreased severity of malaria.

On the basis of these considerations, the authors suggest that the glutathione reductase deficiency detected among Amazonians probably occurred because of a deficiency in the riboflavin derivative FAD. This was due to the fact that this population consumes a peculiar diet, has a high prevalence of helminthic infestation and is subjected to the selective pressure exerted by malaria.

If on the one hand riboflavin deficiency protects individuals against malaria, on the other it makes the erythrocytes quite susceptible to the hemolysis due to oxidative stress caused by drugs. In this case, oxidant antimalarial drugs should be used with caution, since the antioxidant systems of these erythrocytes are impaired. This phenomenon would worsen if any one of these individuals also had a concomitant genetic defect, such as, for example, G6PD deficiency.

Considering this information as a whole, preference should be given to antimalarial drugs that do not have an important oxidant effect on red blood cells to treat patients from endemic regions in which genetical or acquired alterations in erythrocyte metabolism occur.

In this respect, clindamycin, as an antibiotic, acts in a different manner than antimalarial drugs. This drug was first used by MILLER et al. to treat patients with infection caused by *Plasmodium falciparum* resistant to multiple drugs. These authors combined the drug with quinine for 3 days in order to obviate the slow action of the antibiotic in reducing parasitemia. In Brazil, ALECRIM et al. first used clindamycin alone for the treatment of infection by resistant *Plasmodium falciparum* in patients from the Amazon region. Later on PEREIRA et al. and MEIRA et al., working in the township of Humaitá (AM), used clindamycin alone for the treatment of malaria caused by *Plasmodium falciparum*, obtaining good therapeutic results.

The action of this antibiotic on *Plasmodium falciparum* seems to occur by inhibition of the synthesis of function of the parasite's mitochondria, as reported by GEARY et al. in “in vitro” studies. Clindamycin is known to penetrate bacteria and to act by interaction with the 50S subunit of ribosome 70S. DIVO et al. demonstrated that *Plasmodium falciparum* has a single mitochondrion and exhibits a complex growth, development and replication pattern during erythrocyte schizogony, when evidence of metabolic mitochondrial activity has been observed. Since 70S ribosomes virtually identical to those of the bacteria are present in the mitochondria of the Plasmodium cells, the current opinion is that clindamycin may act on the plasmodium exactly at the mitochondrial level.

SEABERG et al. conducted “in vitro” studies on the behavior or chloroquine-resistant and sensitive *Plasmodium falciparum* strains in the presence of different clindamycin concentrations. The authors concluded that the effect of the antibiotic in eliminating asexual parasitemia does not depend on the dose used, but rather on the time of plasmodium exposure to the drug, with growth inhibition occurring after 24 hours of exposure and maximum inhibition after 72 hours.

Since clindamycin does not act rapidly on malarial parasites, prolonged treatment (at least 5 to 7 days) is needed. BARRAVIERA's concern was that the proposed use of clindamycin as an antimalarial drug might cause the same side effects as observed with other antimalarial drugs. Thus, the author studied the effect of clindamycin on G6PD and glutathione
reductase activity in normal red blood cells using the drug concentration recommended for patient treatment, i.e. 20 mg/kg body weight/day. Red blood cells were incubated with the drug and G6PD and glutathione reductase activities were evaluated. No significant difference was observed between groups, showing that the antibiotic did not change NADPH generation in red blood cells, leaving the normal functioning of these enzymes unaffected.

BARRAVIERA et al. also tested G6PD and glutathione reductase activity in a G6PD-deficient Black Amazonian patient with malaria caused by Plasmodium falciparum. This patient was treated with clindamycin according to the schedule recommended by MEIRA et al. (7 days), with good therapeutic results. At the end of treatment, the patient showed slight anemia which was explained by the natural evolution of the disease, with no hemolytic crises detected. It should be pointed out that the bilirubin levels of the patient were within normal limits throughout the observation period. BARRAVIERA et al. tested 31 patients with malaria caused by Plasmodium falciparum for glutathione reductase activity. Eleven of these patients were deficient and were treated with clindamycin as recommended by MEIRA et al., with no hemolytic crises occurring.

On the basis of these considerations, authors have suggested that even though clindamycin has a slow schizonticidal effect, it can be an alternative choice for the treatment of patients with malaria caused by Plasmodium falciparum and with genetic or acquired erythrocyte alterations that render them vulnerable to hemolysis due to oxidative stress. Thus, G6PD-, glutathione reductase-, glutathione peroxidase- and riboflavin-deficient patients will benefit from this type of treatment, which would prevent the hemolysis frequently occurring with the use of other antimalarial drugs. Since clindamycin is not an oxidant drug, it does not induce production of O3 radicals and therefore does not cause oxidative stress on red blood cells as a consequence of drug-induced hemolysis. Because of the characteristics of this antibiotic, the concomitant use of oxidant antimalarial drugs such as quinine, as suggested by some investigators, is permitted without any damage to red blood cells.

REFERENCES


