areas raises the specter of delayed oncogenesis in neighboring soft tissue and has been implicated in the constriction of vessels at the margins of irradiated stents — the so-called "candy wrapper" or "edge" effect. These issues must be resolved with carefully crafted clinical trials, the results of which could justify the evidence-based expansion of indications for a promising therapy for coronary and other vascular disease. Nevertheless, at this time, brachytherapy appears to provide a valuable addition to the armamentarium of the interventional cardiologist.

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A MOLECULAR MARKER FOR CHLOROQUINE-RESISTANT FALCIPARUM MALARIA

MALARIA caused by *Plasmodium falciparum,* a protozoan parasite of the blood, is responsible for up to 2.7 million deaths yearly, mainly in children in sub-Saharan Africa. Worldwide there are over 300 million new cases of malaria per year. Given the lack of a suitable vaccine, the main ways to reduce morbidity and mortality are the use of insecticide-impregnated netting around the bed and chemoprophylaxis for specific groups at increased risk, such as nonimmune travelers to an area where malaria is endemic, pregnant women,[1] and children with sickle cell anemia. However, in many areas the effectiveness of treatment and of efforts to prevent falciparum malaria is being limited by the development of drug-resistant strains.[2]

Chloroquine was introduced in the 1940s and soon became the mainstay of therapy and prevention, because it was cheap and nontoxic and malaria parasites were universally susceptible to it. Resistance developed in Southeast Asia and South America at the end of the 1950s and in Africa by the late 1970s. In spite of its reduced efficacy, chloroquine is still the first-line antimalarial drug in most parts of Africa, both for reasons of cost and because of the widespread prevalence of partial immunity among symptomatic older children and adults, a factor that enhances the effects of the drug. The rates of resistance are increasing and are having a major effect on the efforts to control the morbidity and mortality associated with *P. falciparum* malaria. In Senegal, the emergence of chloroquine resistance over a 12-year period was associated with at least a doubling of the risk of death from malaria in children younger than 10 years of age.[3] The transmission of resistant strains can be facilitated by unsuccessful treatment.[4]

Electron-microscopical analysis of malaria parasites in red cells of mice and monkeys treated with chloroquine shows that the parasite's lysosome, where hemoglobin is digested, is affected first. The link with hemoglobin digestion explains the drug's inactivity against the pre-erythrocytic stage of the parasite in the liver. Ferriprotoporphyrin IX, which is detached from globin during digestion, is normally detoxified in the parasite's lysosome. The weak base chloroquine accumulates in the acidic lysosome[5] and binds to ferriprotoporphyrin IX,[6] thereby preventing its detoxification and thus destroying the parasite (Fig. 1). Resistance to chloroquine was shown to be due to reduced uptake of the drug by the infected erythrocyte[7] and presumably reflects a reduction in the accumulation of the drug in the parasite's lysosome. The slow rate of development of resistance to chloroquine may reflect the host-derived nature of the target, the importance of hemoglobin digestion, and the complex mechanisms required for resistance.

The ability of verapamil, when given in combination with chloroquine, to reverse in part the resistance of strains in vitro parallels the ability of the drug to inhibit multidrug resistance in cancer cells.[8] There are associations between chloroquine resistance and mutations in an *mdr*-like gene (*pfmdr 1*) on chromosome 5 that encodes a protein, Pgh1, located in the lysosomal membrane of the parasite.[9] Field studies showed that in areas with a high prevalence of resistant strains, these mutant alleles could also be found in chloroquine-sensitive parasites and were absent in some resistant strains. Evidence of the involvement of another genetic determinant was found in the progeny of a genetic cross between chloroquine-resistant and chloroquine-sensitive clones, the study of which indicated a link between chloroquine resistance and a locus on chromosome 7.[10] Because both parental clones had mutations in *pfmdr 1,* no primary link to chromosome 5 could be demonstrated, but its involvement could also not be ruled out.

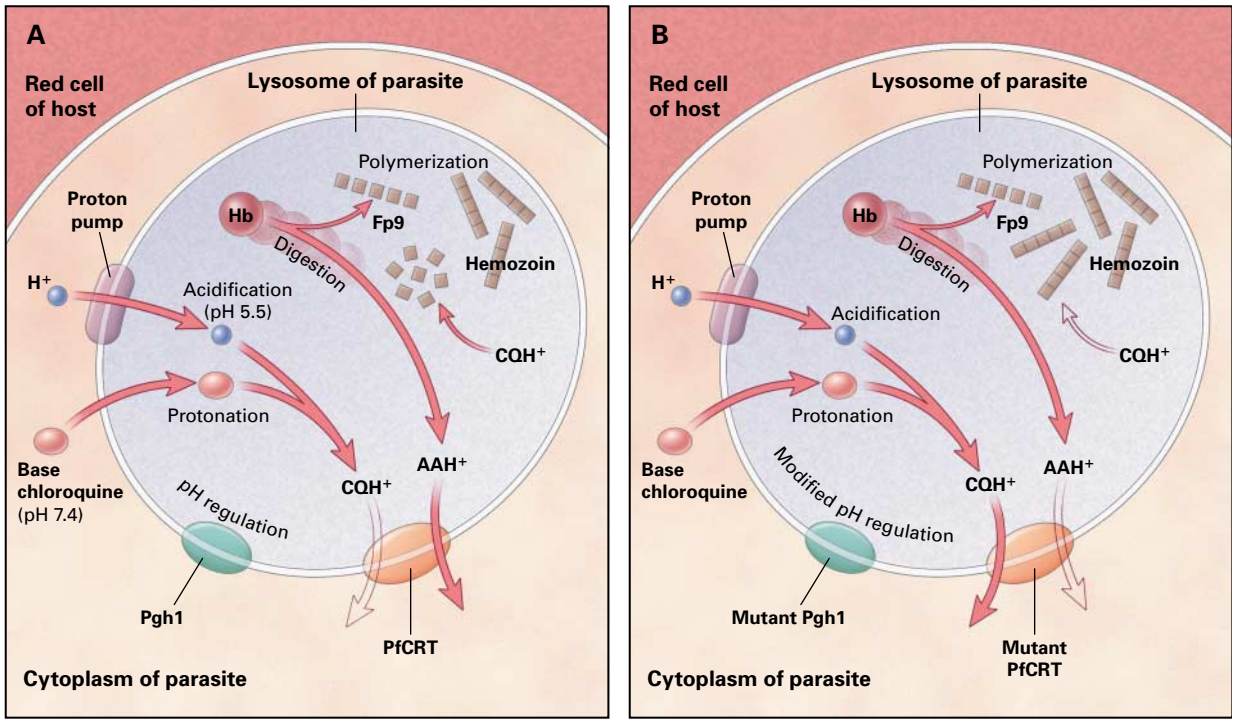

Figure 1. The Effect of Chloroquine on Heme Detoxification in the Lysosome of a Chloroquine-Sensitive *Plasmodium falciparum* Malaria Parasite (Panel A) and a Chloroquine-Resistant Malaria Parasite (Panel B).

In Panel A, in the lysosome of a chloroquine-sensitive parasite, hydrogen ions enter through the proton pump, acidifying the lysosomal environment (pH 5.5). This process is probably regulated by the Pgh1 protein, which releases anions into the lysosome to optimize the difference in the transmembrane charge. During the digestion of hemoglobin (Hb), protonated basic amino acids (AAH^+) are released together with toxic ferriprotoporphyrin IX (Fp9). Ferriprotoporphyrin IX is detoxified by polymerization to crystalline hemozoin. The weak base chloroquine, present in the cytoplasm (pH 7.4), dissolves in the lysosomal membrane and enters the acidic environment, undergoing protonation to a form (CQH^+) that is insoluble in the membrane and that quickly becomes concentrated. CQH^+ binds to ferriprotoporphyrin IX and thus inhibits its polymerization, which leads to the accumulation of ferriprotoporphyrin IX, causing membrane damage. The protonated basic amino acids exit the lysosome by means of the transmembrane protein PfCRT. The PfCRT protein probably has a limited affinity for CQH^+ and exports some of the drug from chloroquine-sensitive parasites.

Panel B shows the lysosome of a parasite with mutations in *pfcrt* and *pfmdr 1* related to chloroquine resistance. The mutant PfCRT probably has an increased affinity for CQH^+ and exports large amounts of the drug, enabling the polymerization of ferriprotoporphyrin IX to proceed normally. Concomitantly, the mutant PfCRT would have a reduced affinity for AAH^+, which may reduce the efficiency of the export of AAH^+ and, in the absence of chloroquine, result in the accumulation of more protons (H^+) in the lysosome. The presence of mutant Pgh1 may partially prevent this accumulation of protons, increasing the fitness of parasites with *pfcrt* and *pfmdr 1* mutations. The mutation in *pfmdr 1* also increases the sensitivity of the parasite to mefloquine and artemisinin, probably as a result of the partial inactivation of the ability of mutant Pgh1 to export these drugs.

The results of extensive laboratory investigations of *pfcrt*, a gene on chromosome 7 that encodes a transmembrane protein, PfCRT, in the lysosomal membrane, have now been reported.[11] The wild-type PfCRT resembles proteins that facilitate the transport of organic cations and may normally result in the efflux of protonated basic amino acids,[12] which otherwise would accumulate in the lysosome. A mutation in *pfcrt* leads to the substitution of threonine (T76) for lysine (K76) at position 76 (K76T) and results in resistance to chloroquine in vitro. Alterations in PfCRT could well reduce the efficiency of amino acid export, and this change could account for the lower pH in the lysosomes of chloroquine-resistant parasites.[11] Transfection of wild-type *pfmdr 1* makes mammalian lysosomes more acidic,[13] and the presence of mutant Pgh1 might allow lysosomal pH to reach a more normal level in a parasite with chloroquine resistance and a tendency to have a lower lysosomal pH as a result of the mutation in *pfcrt*.

The association of mutations in *pfcrt* and *pfmdr 1* with resistance in vivo is the subject of a field study by Djimdé et al.[14] whose results are reported in this issue of the *Journal*. To assess the association between clinical chloroquine resistance and mutations in these genes, the investigators examined drug responses in patients with uncomplicated falciparum malaria in an area of Mali where the disease is endemic. The positive predictive value of *pfcrt* T76 (the percentage of positive tests that correctly predicted a treatment failure) was only 30 percent, but the negative predictive value (the percentage of negative tests that correctly predicted successful treatment) was 98 percent. These results improved appreciably when patients younger than 10 years of age were considered as a separate group. The positive predictive value should be higher in areas with a lower prevalence of endemic disease, in epidemics, and in cases of malaria among travelers to areas where the disease is endemic. Even in the area that Djimdé et al. studied, the results showed that the absence of *pfcrt* T76 was an excellent predictor of successful chloroquine treatment.

The association of other mutations in *pfcrt* with the clinical outcome is not as clear-cut as that between T76 and chloroquine treatment. It appears that this one mutation, which leads to a change in a single amino acid in a transmembrane domain, can provide a basal level of chloroquine resistance and may be accompanied by additional compensatory mutations. Enhanced levels of resistance may also require the presence of a mutation in *pfmdr 1*. In West African isolates, tyrosine at position 86 (Y86) is the mutation of *pfmdr 1* generally seen in association with chloroquine resistance, so the absence of other mutations in these samples is not unexpected. That *pfmdr 1* has an ancillary role is emphasized by the observation that though Y86 was sometimes seen in the absence of *pfcrt* T76 in the samples obtained before treatment, it was not seen alone in samples obtained from patients in whom treatment was not successful.

Although the prevalence of *pfcrt* T76 in the samples obtained before treatment was only 41 percent, the prevalence in samples obtained from patients with persistent or recurrent infection after treatment (14 percent of the total study population) was 100 percent. The respective values for *pfmdr 1* Y86 were 50 percent and 86 percent, indicating selection for this allele after chloroquine treatment, as reported earlier.[15] In contrast to the results for *pfcrt*, 30 percent of the samples obtained from patients with persistent or recurrent infection carried the wild-type allele at position 86 of *pfmdr 1*, either alone (14 percent) or in combination with the mutant allele (16 percent).

That the *pfmdr 1* Y86 mutation is important is shown by the increase in specificity (the percentage of successful treatments that were correctly predicted on the basis of a negative test) from 63 percent when *pfcrt* T76 was considered alone to 78 percent when *pfcrt* T76 and *pfmdr 1* Y86 were considered together. In laboratory transfections,[16] the presence of mutant *pfmdr 1* enhanced resistance of clones only when there was already a low level of chloroquine resistance (presumably these clones carried the *pfcrt* T76 allele).

These observations suggest that a mutation in *pfcrt* is required to confer a basic level of resistance before mutations in *pfmdr 1* can have an effect. The presence of both mutations may, however, be responsible for the finding that in 13 percent of the resistant infections, the level of drug resistance was class II or III (i.e., a level indicating persistent parasitemia). A rapid, sensitive test to detect *pfcrt* T76 in blood samples may be useful in the treatment of infected patients, since a negative result would indicate the probable effectiveness of treatment with chloroquine alone. Cost considerations in many areas would support the use of empirical treatment with chloroquine or another alternative first-line drug, such as sulfadoxine–pyrimethamine, in patients with uncomplicated malaria, at least in adults. In the case of more expensive drug regimens, the cost of the test would have to be balanced against the potential saving in the costs of treatment. Of more immediate value is the fact that screening an infected population for the presence of *pfcrt* T76 could detect the arrival of resistance and, with regular surveillance, could be used to monitor its prevalence. The results could help influence recommendations for the first-line treatment of malaria. Knowledge of the initial mutation in chloroquine resistance also has major implications for the design of rational alternatives to the widely used 4-aminoquinolines.

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Predicting Cerebral Edema during Diabetic Ketoacidosis

CEREBRAL edema is a devastating complication of diabetic ketoacidosis and remains the leading cause of serious illness and death in children with diabetes mellitus.[1] The causes of cerebral edema are unknown, largely because of the lack of large-scale population-based studies. However, several hypotheses relating to possible antecedent risk factors and the effects of various treatment regimens have been proposed.[1]

In this issue of the *Journal,* Glaser et al.[2] report the results of a retrospective analysis of cases of children with diabetic ketoacidosis at 10 centers. Among 6977 such children, the authors identified 61 with cerebral edema. The incidence of cerebral edema was 0.9 percent, a rate remarkably similar to that reported historically in the United States[3] and recently in the United Kingdom.[4] As in previous studies, the risk was highest among younger children with newly diagnosed diabetes.[1] The 21 percent mortality rate associated with cerebral edema was similar to that in the United Kingdom,[4] as was the morbidity rate, with a substantial 27 percent of the survivors having neurologic sequelae.

The development of cerebral edema may be the result of the treatment that children receive for diabetic ketoacidosis; treatments such as high doses of insulin and the administration of bicarbonate or large volumes of hypotonic fluid would be the major culprits.[1] However, it is also possible that the condition is an idiosyncratic response to diabetic ketoacidosis. Thus far, no adequate proof for either hypothesis has been advanced.

The study by Glaser et al. confirms previous reports that cerebral edema can become evident even before treatment for diabetic ketoacidosis is initiated, but usually it develops 4 to 12 hours later.[1,2] The only treatment association detected by the authors was with the administration of bicarbonate; other treatments that previously have been implicated,[1] such as a high rate of fluid administration or the administration of hypotonic fluid, were not associated with an increased risk of cerebral edema. Nevertheless, a smaller increase in the serum sodium concentration during therapy was associated with cerebral edema, as suggested in previous reports.[1,5] However, as the authors state, the failure of serum sodium concentrations to rise substantially could be a consequence, rather than a cause, of cerebral edema.[2]

Glaser et al. are to be commended for their systematic study, but does it improve our understanding of the pathophysiology of cerebral edema or provide new guidelines for therapy? The authors note that cerebral edema was associated with higher serum urea nitrogen concentrations and lower partial pressures of arterial carbon dioxide at the time of diagnosis; they argue that this finding supports the hypothesis, first proposed by Dillon et al.,[6] that cerebral edema is the result of reduced blood volume, aggravated by a reduced partial pressure of arterial carbon dioxide and leading to cerebral vasoconstriction, cerebral ischemia, and hypoxia. The reduced partial pressure of arterial carbon dioxide at presentation in children with cerebral edema may imply an increased respiratory drive in response to acidosis. However, caution must be used in interpreting blood pH values, partial pressures of arterial carbon dioxide, and bicarbonate concentrations when corrections have been made to account for the collection of both arterial and venous blood samples. Furthermore, whereas rapid reductions in the partial pressure of arterial carbon dioxide may lead to acute vasoconstriction, this process is often reversed when low partial pressures are chronic, as in cases of adaptation to metabolic acidosis. However, the hypoxia theory has received support from those who have suggested that bicarbonate therapy might reduce the partial pressure of oxygen in the cerebrospinal fluid, leading to vasoconstriction and further brain hypoxia.[7]

The higher initial serum urea nitrogen concentrations noted by Glaser et al. in children in whom cerebral edema developed (and, by inference, the greater degrees of renal insufficiency in these children) have been linked to pure anion-gap acidosis, rather than hyperchloremic acidosis.[8] The type of acidosis may therefore be important in predicting cerebral edema, since regulation of cell volume by the sodium–hydrogen ion exchanger may be compromised by severe acidosis.[9] The higher serum urea nitrogen concentrations in these children may reflect the chronic nature of diabetic ketoacidosis, because a long duration of symptoms has previously been associated with development of cerebral edema.[3]

The data presented by Glaser et al. confirm previous reports that the administration of bicarbonate may be a risk factor for cerebral edema in children with diabetic ketoacidosis; however, over the past 10 years,