**Plasmodium** phospholipid metabolism: a target for the development of novel antimalarial drugs

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A programme for developing new drugs for the treatment of *Plasmodium falciparum* malaria is targeted against the essential phospholipid metabolism of the intra-erythrocytic stages of the parasite. Blockage of the choline transporter that provides the intracellular parasite with choline, a precursor required for synthesis of phosphatidylcholine, the major phospholipid of the parasite, seems to hold the most promise. Molecules with the ability to interfere with this step, whose structures have been optimised using structure-activity criteria, have been synthesised. The antimalarial activity of the compounds produced so far, which have in-vitro activities in the ng/ml or nanomolar ranges, appears satisfactory. The present compounds are also active, in vitro, against parasites resistant to the antimalarial drugs already in clinical use. In vivo, one of the compounds, G25, successfully cleared high parasitaemias of murine parasites in mice and of *P. falciparum* in *Aotus* monkeys. There were no recrudescences in the treated monkeys and the therapeutic index of the drug in monkeys is probably >50. Targeting of phospholipid metabolism therefore appears to be a rational and promising approach to the development of new antimalarial drugs.

The increasing prevalence and range of resistance to conventional antimalarial drugs in the parasites causing human malaria mean that new antimalarial compounds need to be developed. Thorough biological and biochemical studies of the parasites could lead to the discovery of a specific target that could be used in the design of drugs capable of exterminating the parasite without injuring the host. In one such design programme, the target is the essential phospholipid (PL) metabolism developed by *Plasmodium* spp.
during their intra-erythrocytic cycles. This metabolism, which does not occur in mature, uninfected mammalian erythrocytes, is very intense, the PL content of an erythrocyte increasing by as much as 6-fold following infection. The large quantity of PL produced by the parasite is needed for the biogenesis of membranes. PL metabolism is vital for the parasite, since interfering with this metabolic pathway leads to parasite death.

To synthesise its PL, the parasite possesses a variety of complex metabolic pathways but depends on the host for supplies of the polar head groups and fatty-acid molecules which it cannot synthesise itself. Of all the steps in the pathway, that of choline transport seems the most promising target. The choline transporter provides the parasite with a supply of precursors for the synthesis of phosphatidylcholine (PC), the major PL of infected erythrocytes. One transport and three enzymatic steps occur between extracellular choline and its incorporation into PC. Each of these steps has now been characterized. The action of one enzyme, choline phosphate cytidylyltransferase, which has just been cloned and sequenced, is a rate-limiting step. However, choline transport, which also regulates and limits the supply of extracellular choline to the parasite, is also a crucial step in the pathway and, being extraparasitic, is very accessible. Choline transport was therefore chosen as a prime pharmacological target. Choline entry into the erythrocyte is carrier-mediated. After infection of the cell with a malarial parasite, although entry remains totally controlled and the carrier has a similar affinity for choline as before, choline entry increases 10-fold.

More than 350 compounds targeted against PL metabolism have now been synthesised, by two groups of chemists, and tested against the growth of P. falciparum. Interfering with the transport of the polar head, choline, has been shown to be lethal to the parasite. Forty of the compounds have been found to possess significant antimalarial activity in vitro, not only against generally sensitive strains of the parasite but also against multidrug-resistant isolates, with median inhibitory concentrations (IC50) of < 80 nM. When > 100 isolates from Cameroon were tested with one of the compounds, G25, no cross resistance was observed between G25 and four standard, antimalarial compounds but sensitivity to G25 was inversely correlated to sensitivity to quinine.

In vivo, 10 of the new compounds possessed effective antimalarial activity against P. chabaudi. The therapeutic index [the ratio of the median lethal dose for the mice (LD50) to the median effective dose for the parasite (ED50)] for each of the 10 compounds varied between 8 and 40. The antimalarial activity of each compound is very specific to mature parasites (trophozoites), as determined with P. falciparum and P. berghei in vitro and with P. chabaudi in vivo. This specificity corresponds to the most intense phase of phospholipid biosynthesis during the parasite cycle, thus corroborating the mechanism of action. (The 10 compounds, at similar concentrations, also possess in-vitro and in-vivo activity against Babesia.)

When G25 was tested against the sexual stages of P. berghei in vitro, at concentrations up to 10 nM, it was not found to have any effect on the maturation of gametocytes. However, G25 inhibited zygote development into the mobile ookinete, indicating that the compound's target is operative at this stage and might be essential for ookinete development.

In vivo, when administered intraperitoneally twice daily and investigated by a modified 4-day suppressive test, G25 was found to be very active against P. chabaudi (ED50 = 0.06 mg/kg) and only slightly less active against P. vinckei petteri. Plasmodium berghei, however, was much less sensitive than these other murine parasites in vivo (3- to 20-fold, depending on the strain). (Plasmodium yoelii also appeared relatively insensitive to G25.) These differences in activity could result from variation in the degree of synchronism in every strain, variation in their preferred host-cell type at invasion (i.e. mature or immature erythrocytes) or from an intrinsically lower sensitivity of the P. berghei strain to G25. In the P. chabaudi-mouse model, G25 fully succeeded in inhibiting para-
sitaemia which had already reached 11%, without any decrease in its therapeutic index.

In vivo, when administered at 0.2 mg/kg intramuscularly twice a day for 8 days to Aotus monkeys infected with *P. falciparum*, G25 fully succeeded in curing parasitaemia which had reached 6%, without recrudescence (as checked by PCR), showing it to be as effective as quinine or sulphadoxine–pyrimethamine in curing high parasitaemias in the monkey. Complete curative antimalarial activity, without recrudescence, was obtained with doses as low as 30 μg/kg. At 10 μg/kg, G25 was active (i.e. the monkey was cleared of parasitaemia) but not curative, as recrudescence occurred. The therapeutic index for G25 in the monkey appears to be >50. The biphasic parasitaemias observed in two of the 10 monkeys investigated probably reflect the threshold of parasite sensitivity to G25.

Development of the present pharmacological model has thus been fully validated in malaria-infected mice and monkeys, notably in the curing of high parasitaemias without recrudescence and with an acceptable therapeutic index. However, G25 is poorly absorbed through the gastro-intestinal tract. Recently, 35 new compounds of the M and MS series, which are bioisosteric analogues of G25, have been synthesised. Like G25, they possess potent antimalarial activity against *P. falciparum in vitro* (all with IC50 in the nanomolar range, and 10 with IC50 of <10 nM). The new leading compounds, M64 and MS1, are not only better tolerated than G25 (by 20-fold) but also far better absorbed (by 7- to 15-fold), as seen in the ratio of the LD50 after intraperitoneal and oral administration. Although both are active against *P. vinckei* peseri in mice, with therapeutic indexes of about 7, their activities against *P. falciparum* in Aotus remain to be evaluated. Ex-vivo tests, to measure, using bioassays, the serum concentrations of the drugs in mice, dogs and monkeys which each received a single dose of G25 or MS1, have also been conducted. The drugs were given by intramuscular (all animals), oral (all animals) or rectal routes (dogs only). The compounds were always rapidly distributed, with a distribution half-life of <1 h in all of the animals. In mice, elimination half-lives were only 6 h for G25 but 11 h for MS1, whichever the route of administration. The results of the tests using dogs were surprising, elimination being extremely rapid (with a half-lives of <1 h for G25 and <2 h for MS1), whereas the rates of elimination from the monkeys were similar to those observed in mice. The rapid elimination of both compounds from the dogs probably reflects their rapid metabolism. As administration of the drugs by the rectal route led to similar maximal serum concentrations of the compounds (or of their active metabolites) as similar doses given orally, the potential for rectal administration of the compounds must not be ignored. Even when MS1 was administered to monkeys at a sixth of the lethal dose, the subsequent, maximal concentration in the blood was about 40 ng/ml (i.e. 80-fold higher than the IC50 against *P. falciparum in vitro*). If maximum plasma concentrations of the drug and elimination rates are considered together, it becomes clear that the plasma concentration of MS1 will be above the IC50 for 80 h after just a single dose of 5 mg/kg. Based on these results, a treatment schedule of one administration per day should result in adequate plasma concentrations.

In conclusion, the rationale behind the present, drug-development programme was to block the development of the parasite using drugs which selectively interfere with phospholipid metabolism. Impairing PL metabolism by use of choline analogues is an original strategy. The antimalarial activities of the compounds developed and investigated so far are satisfactory, with in-vitro activity in the ng/ml or nanomolar range. G25 has a preferential effect on the trophozoite form of the parasite, taking <5 h to kill the mature stages. Many of the compounds investigated are active, in vitro, against parasites resistant to the antimalarial drugs in clinical use, demonstrating their potential efficacy in areas of multidrug resistance. In vivo, against murine parasites in mice or against the human parasite *P. falciparum* in monkeys, G25 successfully cleared high parasitaemias. This
compound cleared parasitaemias and prevented recrudescences of *P. falciparum* in *Aotus* monkeys, with a therapeutic index probably $>50$. The active compounds investigated are inexpensive to produce, stable and water soluble. Further investigation of the current leading compounds and other compounds produced against PL metabolism now seems fully justified.

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