J. Pharm. Pharmacol. 1997, 49 (Suppl. 2): 59–64 © 1997 J. Pharm. Pharmacol.

The Treatment of Malaria with Iron Chelators

ROBERT C. HIDER AND ZUDONG LIU

Department of Pharmacy, King's College London, Campden Hill Road, London W8 7AH, UK

New routes to chemotherapeutic attack on malaria are urgently required as drug resistant mutants to the antifolate pyrimethamine and the quinine family are becoming widespread. Without new drugs, falciparum malaria could become an untreatable disease in some regions within the next decade.

The idea that iron chelation might have antiplasmodial effect emerged from the awareness that many microbial pathogens depend on host-derived iron for virulence. In 1982 it was demonstrated by Pollack and co-workers that desferrioxamine possessed an antiplasmodial effect in-vitro (Raventos-Suarez et al 1982). Some years later this was followed by a clinical trial of iron-chelation therapy in children with cerebral malaria (Gordeuk et al 1992a). Desferrioxamine quickly reduced the symptoms of parasitaemia. Thus it is possible that iron chelation offers a novel approach to controlling malaria infections.

The Biology of the Erythrocyte-bound Stage

The schizont exists in the erythrocyte and it is this stage which is severely influenced by iron chelation. Merozoites released from the hepatic schizonts or from asexual blood-stage schizonts interact with uninfected red cells (erythrocytes). The invasion is highly specific, the parasite producing a parasitophorous vacuole membrane (PVM) which is continuous with the red cell membrane. Once surrounded by the PVM the parasite becomes encapsulated within the red cells cytosol (Fig. 1) (Pasvol et al 1992).

The membrane of the parasitized red cell differs in many respects from that of the parent membrane and presumably the new parasite proteins and lipids are introduced via the PVM while it remains fused to the original red cell membrane. The parasite (schizont) possesses a high metabolic activity, in contrast to the host erythrocyte and therefore nutrients need to be acquired and waste products removed. The rate of glycolysis can be up to 50 times that of the uninfected erythrocyte. The growing parasite degrades glucose, almost quantitatively, to lactate and, as the tricarboxylic acid cycle is absent, lactate is not oxidized further and has to be excreted. Several transport channels and proteins are located in the erythrocyte membrane which are not present in the uninfected cell (Cabantchik 1989; Elford et al 1996). The function of these proteins is to facilitate the uptake of nutrients and efflux of waste materials, a classic example being that of lactate efflux. These facilitative transport systems offer routes for selectively targeting chemotherapeutic agents including iron chelators.

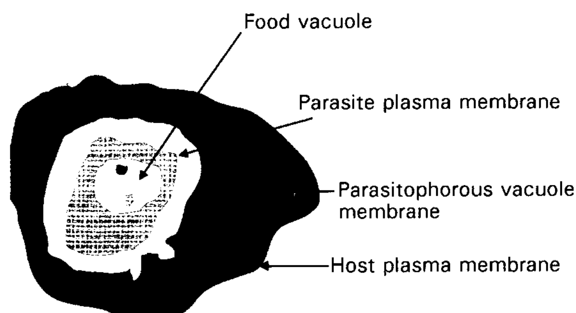


FIG. 1. Malaria parasite in the trophozoite stage. Pharmaceuticals have to permeate three membranes to gain access to the cytoplasm of the parasite.

Correspondence: R. C. Hider, Department of Pharmacy, King's College London, Campden Hill Road, London W8 7AH, UK.

The parasite, once engulfed in the erythrocyte, develops into the trophozoite stage, and uses host haemoglobin as a major nutrient source. Access to haemoglobin is achieved via the bridging structure, the cytosome. Haemoglobin-containing vesicles are pinched off from the cytostome and fuse with digestive vacuoles. Degradation occurs in these vacuoles at the pH range 5·0–5·5, the process being initiated by an aspartic proteases which clip the intact haemoglobin molecule. The resulting polypeptides are then hydrolysed to their constituent amino acids by parasite-specific hydrolysis (Goldberg et al 1990). During this process haem is released and accumulates as crystalline particles (haemozoin). Haemozoin is a polymer of haem groups linked between the central ferric iron of one haem and the carboxylate side group of another. The parasite thereby avoids the toxicity normally associated with soluble haem groups. Between 25 and 75% of the haemoglobin in an erythrocyte is degraded in this manner. Thus, with a serious malaria infection (20% parasitaemia), up to 100 g of haemoglobin is used during each cycle. These events continue for about 36 h leading to pigmented trophozoites which almost fill the erythrocyte. A phase of multiplication (schizogony), follows when the nucleus of the parasite undergoes repeated divisions, portions of the cytoplasm aggregating around the daughter nuclei to form up to 24 merozoites. After a further 48 h the envelope of the exhausted erythrocyte bursts and the merozoites are liberated into the plasma, where they attack fresh erythrocytes and the cycle is repeated.

Iron is essential for parasitic growth and multiplication, a particularly critical enzyme being ribonucleotide reductase, which is an iron-containing metalloenzyme essential for DNA synthesis (Stubbe 1990). Thus if iron-specific chelators can be selectively directed to infected erythrocytes, then a new form of chemotherapy becomes possible.

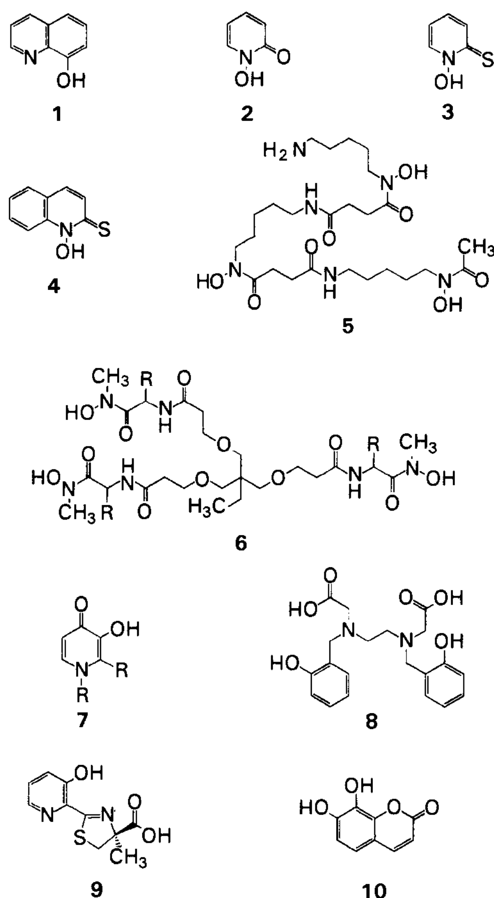
Iron Chelators as Antimalarials

Adrien Albert was the first to realize the potential of iron chelators as chemotherapeutic agents and over the period 1945–1955 he produced much data indicating the potential of bidentate ligands such as 8-hydroxyquinoline (**1**), 1-hydroxy-

Table 1. Inhibition of *P. falciparum* by iron chelators.

Compound	ED50 (M)
8-Hydroxyquinoline (**1**)	$8{\cdot}3 \times 10^{-9}$
2-Methyl-8-hydroxyquinoline	$1{\cdot}2 \times 10^{-6}$
5-Methyl-8-hydroxyquinoline	$3{\cdot}1 \times 10^{-8}$
1-Hydroxypyridin-2-thione (**3**)	$7{\cdot}9 \times 10^{-10}$
1-Hydroxyquinolin-2-thione (**8**)	$1{\cdot}3 \times 10^{-7}$

ED50 is the concentration required to reduce in-vitro growth of *P. falciparum* by 50% after exposure for 3 days (Scheibel & Adler 1980, 1982)



pyridin-2-one (**2**) and 1-hydroxypyridin-2-thione (**3**) (Albert et al 1956) (Table 1). Albert pointed out that potent antibacterial molecules required a high stability constant for iron (III) and a high partition coefficient to facilitate cell penetration; he postulated that the 1 : 1 iron/ligand complex was toxic. This concept was applied to *P. falciparum* by Scheibel and Adler in the early 1980s (Scheibel & Adler 1982) when they considered a range of 8-hydroxyquinoline and 1-hydroxypyridin-2-thione analogues, together with diethyldithiocarbamate. As a result of these studies, compounds with similar in-vitro potency to that of quinine were identified, for instance 1 hydroxyquinolin-2-thione (**4**). A summary of these early results is presented in Table 1. Significantly the smallest compounds (**1**) and (**3**) are the most potent, a finding which contrasts with the bacteriostatic properties of the same series, where lipophilicity is critically important, the more lipophilic ligands being the most toxic (Scheibel & Adler 1982). Significantly there is a much larger drop in inhibitory properties of 8-hydroxyquinoline when a methyl function is introduced close to the chelating centre, namely 2-methyl in contrast, to 5-methyl, the latter inducing only a slight reduction (Scheibel & Adler 1980). These results are best interpreted as the ligands exerting their activity by inhibiting a critically important metalloenzyme.

Following this work, it soon became apparent that hydrophilic iron chelators also possess antimalarial activity under both in-vivo and in-vitro conditions, this series of studies being pioneered by Raventos-Suarez et al (1982). Desferrioxamine (**5**) was found to possess an ED50 value of 10 μM and yet possesses a low K_{part} value. Furthermore desferrioxamine was found to induce a powerful stage-dependent inhibitory effect. Table 2 shows hypoxanthine incorporation, as a measure of growth by parasites. Exposure to desferrioxamine in 8 h pulses during the first 23 h, when the parasites were between ring and non-pigmented trophozoite stages had no inhibitory effect on growth. In contrast exposure to desferrioxamine after 23 h, when the parasites had matured into pigmented trophozoites and schizonts, resulted in more than 50% supression of hypoxanthine incorporation (Whitehead & Peto 1990). Thus sensitivity to iron chelation occurs late in the parasite cycle and coincides with the period of maximum DNA synthesis. This renders ribonucleotide reductase as a likely target for inhibition by hydrophilic iron chelators. A wider variety of iron chelators are known to inhibit ribonucleotide reductase (Ganeshaguru et al 1980; Cory et al 1981).

Subsequent to these key investigations, a diverse range of iron chelators has been demonstrated to possess antimalarial activity. These include: *tris*-hydroxamate siderophores including tripodal hydroxamates (**6**) (Shanzer et al 1991; Lytton et al 1993); 3-hydroxy-pyridin-4-ones (**7**) (Heppner et al 1988; Hershko et al 1992); phenolic and catecholic ligands including HBED (**8**) (Yinnan et al 1989); pyridoxal betaines (Tsafack et al 1996); desferrithiocin (**9**) (Fritsch et al 1987); and daphnetin, a dihydroxycoumarin (**10**) (Yang et al 1992).

Significantly, extracts from the bark of ash trees, which are rich in coumarins have been used as folk remedies to treat malaria in China (Steck 1971). Daphnetin is currently being used clinically in China to treat Bruger's disease, a coagulation disorder (Li 1986).

Mode of Action of Antimalarial Iron Chelators

There is no clear consensus of the mode of action of iron chelators and this is almost certainly because there are several potential toxic pathways associated with the molecules. It is

Table 2. [^{3}H]Hypoxanthine uptake by *P. falciparum* following exposure to desferrioxamine.

Exposure time (h)	Hypoxanthine uptake at 42 h (% control)
0–7	100
7–15	84
15–23	102
23–30	51
30+	37
0–42	28

The concentration of desferrioxamine was 100 μM. N = 6 (Peto & Thompson 1986).

likely that chelators with different physical and chemical properties possess different modes of action, relevant properties being: hydrophobicity; size and stereochemistry in the immediate vicinity of the chelating function; redox stability of ligand; and redox stability of iron complex.

Deprivation of parasite iron source

The precise iron source for the malaria parasite is unknown but it has been clearly demonstrated not to be of extracellular origin for *P. falciparum* (Peto & Thompson 1986). The growth rate of the parasite is not influenced by the iron status of the host. When parasites invade erythrocytes they generate a low molecular weight iron pool (Hershko & Peto 1988) which must originate from haemoglobin digestion. Exposure to powerful iron chelators would limit the size of this pool, thereby reducing the rate of incorporation of iron into critical redox proteins and metalloenzymes. This mechanism is likely to be associated with the larger water soluble hexadentate chelators, for instance desferrioxamine (**5**), synthetic trihydroxamates (**6**) and HBED (**8**). Some of the more water soluble bidentate ligands, for instance the 3-hydroxypyridin-4-ones (**7**) may also operate via this mechanism. Control of membrane permeation is a key factor in the efficacy of such molecules and this has been systematically investigated by Lytton et al (1993) with hexadentate ligands and Hershko et al (1992) with bidentate ligands. Both groups found a close relationship between the ability to penetrate the infected erythrocyte membrane by simple diffusion and efficacy of the antimalarial effect. The assignment of this mode of action to desferrioxamine is consistent with its proven ability to inhibit ribonucleotide reductase, there being an appreciable time lag between the entry of the cell by desferrioxamine and inhibition of the enzyme (Cooper et al 1996).

Activation of haemozoin-iron

The stable crystalline structure of haemozoin is critically important for the viability of the parasite, the dissociated haem being potentially toxic due to the generation of hydroxyl radicals under aerobic conditions. Van Zyl et al (1993) proposed that desferrioxamine somehow disrupts the haemozoin complex, thereby generating toxic levels of the dissociated haem moieties. More direct evidence is required for this interesting proposal. Significantly the highly effective peroxide-containing antimalarial artemisinin is also reported to be dependent on haemozoin (Meshnick et al 1993). The polymeric haem is believed to activate artemisinin yielding toxic radicals. It should be noted however that both desferrioxamine (**5**) and 3-hydroxypyridinones (**7**) inhibit the antimalarial activity of artemisinin (Meshnick et al 1993). It is conceivable therefore that the selectivity of artemisinin for infected erythrocytes is not associated with the presence of haemozoin, but rather the elevated level of low molecular weight iron complexes.

Direct generation of hydroxyl radicals due to the presence of a toxic iron complex

This mechanism dates back to Albert (Albert et al 1956) and undoubtedly will occur with the hydrophobic bidentate ligands such as the substituted 8-hydroxyquinolines and possibly extremely hydrophobic 3-hydroxypyridin-4-ones (**7**). Unfortunately it is unlikely that this mode of action will be parasite-selective, highly potent molecules also being toxic to the host. In contrast, hexadentate compounds such as desferrioxamine (**5**), HBED (**8**) and the synthetic tris-hydroxamates (**6**) will not generate hydroxyl radicals in the presence of iron (III) as they form extremely stable non toxic complexes.

Direct inhibition of metalloenzymes

There are a number of important iron-containing enzymes which are potential targets for iron chelation. In general chelating agents are unable to form stable ternary complexes or remove the iron from haem-containing and iron-sulphur enzymes. In contrast enzymes with iron co-ordinated to oxygen ligands appear to be more susceptible, for instance ribonucleotide reductase and lipoxygenase. As the malaria parasite is a rapidly proliferating organism, particularly at the late trophozoite stage, optimal activity of ribonucleotide reductase is essential for DNA synthesis.

The iron centre of this enzyme is co-ordinated directly to the polypeptide backbone and is located approximately 5Å distant from the essential tyrosine radical. The crystal structure of the *Escherichia coli* enzyme demonstrates that the iron centre is not readily assessable to the surrounding aqueous phase (Nordlund et al 1990). Consequently, it is likely that only small, non bulky ligands can gain access to the iron centre. 3-Hydroxypyridin-4-ones (**7**) and 8-hydroxyquinoline (**1**) together with the 1-hydroxypyridin-2-ones (**2**) and -thiones (**3**) are likely candidates.

The influence of 3-hydroxypyridin-4-ones on the cell cycle has been intensively studied by Porter and co-workers (Hoyes et al 1992). By inhibiting ribonucleotide reductase these ligands reversibly inhibit proliferating cells in the S-phase. Removal of the chelator after brief exposure leads to the complete recovery of cells. In addition to this indirect evidence for inhibition of ribonucleotide reductase, ESR measurements of the tyrosine radical signal confirms such inhibition. Indeed, in contrast to desferrioxamine, 3-hydroxypyridin-4-ones inhibit this enzyme extremely rapidly (Cooper et al 1996), which is indicative of a direct inhibition, either by ternary complex formation, removal of the essential iron at the active site of the enzyme, or by quenching the tyrosine radical. The former is more likely, in so far as the inhibitory effect induced by the pyridinones can be rapidly reversed (Hoyes et al 1992).

Use of Desferrioxamine in Man

Desferrioxamine is the only pharmaceutical which is available for the treatment of iron-overload (Porter 1989). As a result, it is widely prescribed for transfusion-dependent thalassaemic patients. Due to its poor ability to penetrate mammalian cells, it is relatively non toxic. However, in contrast to normal erythrocytes, desferrioxamine is capable of entering parasitized cells (Scott et al 1990) and therefore does have potential for limiting parasite proliferation.

As a result of the finding that desferrioxamine given by constant subcutaneous infusion suppresses parasitaemia in *P. falciparum*-infected *Aotus* monkeys (Pollack et al 1987), Gordeuk and co-workers undertook a small clinical trial in Zambia (Gordeuk et al 1992b). Desferrioxamine was administered at a rate of 100 mg kg^{-1}/24 h by continous 72 h subcutaneous infusion to 28 volunteers with asymptomatic *P. falciparum* infection in a randomized, double-blind, placebo-

controlled cross-over trial. A significant decrease in concentration of asexual intraerythrocyte parasites was induced by desferrioxamine. However parasitaemia recurred in the majority of the volunteers over the following 6 months. A parallel study was undertaken in Thailand using an identical dose (Bunnag et al 1992), but with half the patients suffering from symptomatic *P. vivax* infection and the other half from uncomplicated *P. falciparum* infection. In both groups desferrioxamine reduced the parasitaemia to zero within 106 and 57 h and with fever clearance times of 55–60 h, respectively. Again parasitaemia recurred in the majority of patients.

The results of these two studies are summarized in Table 3. Clearly the intravenous route is more effective than the subcutaneous route. The likely explanation is the achievement of higher serum concentrations with intravenous than with subcutaneous administration, the respective concentrations being 7 and 20 μM. The latter value is close to the ID50 value for desferrioxamine against *P. falciparum* determined in-vitro (Mabeza et al 1996). The influence of desferrioxamine on cerebral malaria has also been investigated by Gordeuk and coworkers (1992a). Unrousable coma in severe *P. falciparum* infections is associated with mortality of about 15% in children and 20% in adults (much higher mortality rates are recorded in Africa). In fatal cases the cerebral capillaries and venules are packed with erythrocytes containing malaria parasites, which adhere to the vascular endothelium. The brain becomes ischaemic. A double-blind placebo-controlled study of desferrioxamine (100 mg kg^{-1} $(24\ h)^{-1}$, i.v.) in 83 Zambian children suffering from severe falciparum malaria and all being treated with a standard quinine-based regime was undertaken (Gordeuk et al 1992a). Recovery of full consciousness was more rapid with desferrioxamine and the rate of clearance of parasites was also enhanced in the presence of the chelator. Among 50 children with deep coma, the estimated median recovery times were 68 h with placebo and 24 h with desferrioxamine. In addition to the possibility that desferrioxamine possesses a direct antiplasmodial action, the chelator could have a protective effect on cerebral tissue. Hydroxyl radical damage occurs under ischaemic conditions and iron-induced radicals enhance this damage. Iron chelation with desferrioxamine might therefore be expected to reduce such activity (Mabeza et al 1996).

Table 3. The influence of desferrioxamine (100*mg*kg^{-1}) on parasitaemia in patients infected with two different Plasmodium.

	Time to reduce parasitaemia (h) 50%	90%	100%	n of study	Country
P. falciparum					
Desferrioxamine (s.c.)	28	39	104	16	Zambia
Desferrioxamine (i.v.)	9	25	48	14	Thailand
P. vivax					
Desferrioxamine (i.v.)	17	39	72	14	Thailand

Mabeza et al (1996).

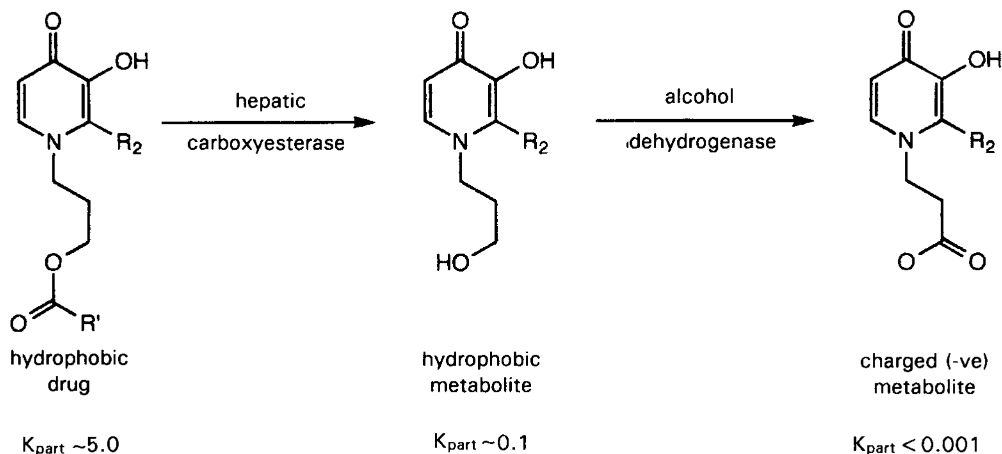
It is clear from the studies that iron chelators have the capacity to clear parasites under clinical conditions. Unfortunately desferrioxamine is not orally active (Hider et al 1994).

Recrudescence

As indicated by the clinical studies with desferrioxamine there is apparently quite extensive recrudenscence. This probably results from the chelator blocking cell cycling of the parasite but not causing death. This particular aspect of iron chelator activity has been studied in detail by Cabantchik and coworkers (Golenser et al 1995; Cabantchik et al 1996). Irreversible damage can be induced in the malaria parasite by the simultaneous use of two chelators, typically one being hydrophilic and the other hydrophobic and therefore highly permeable to the parasite. It is argued that by adversely influencing iron supply to all the three stages, parasite death occurs (Golenser et al 1995) and therefore recrudence will be absent. A successful synergy was observed in-vitro for desferrioxamine and certain tripodal tris hydroxamates (Golenser et al 1995).

Possible Design for Orally Active Iron Chelators

There are several critical features of an iron chelator which endow it with good oral absorption, and these have been recently reviewed (Hider et al 1994). Basically, the molecular weight should be less than 250 and ideally the ligand should be neutral. Thus bidentate ligands of the type (**1**, **2**, **3** and **7**) are ideal for oral absorption. Of these, the 3-hydroxypyridin-4-ones have been most extensively investigated (Porter et al



Scheme 1. Prodrug approach to antimalarial agents.

1990; Dobbin et al 1993). Unfortunately many of the molecules with good antimalarial activity are also toxic to mammalian cells (Hershko et al 1992). The one exception so far tested is CP38 (7, $R_1 = CH_2CH_2CO_2H$, $R_2 = CH_3$) which is an inhibitor of *P. falciparum* but relatively non-toxic to mammalian cells. The partition coefficient (water/n-octanol) for CP38 is extremely low endowing the molecule with poor ability to penetrate membranes (Porter et al 1988) and therefore it is likely that CP38 enters parasitized erythrocytes via a carrier-mediated mechanism, possibly the lactate carrier (Kanaani & Ginsburg 1991, 1992). Thus it may prove possible to specifically target 3-hydroxypyridin-4-ones to the malaria parasite.

In support of the proposal that the anionic CP38 penetrates the infected erythrocyte by facilitated diffusion, is the finding that the property is limited to a well defined structure. Thus in a study of the ability to inhibit the growth of *P. falciparum* by a range of anionic hydroxypyridinones, only CP38 and CP110 possessed ED50 values in the range of 50 μM (Table 4).

Table 4. Structure and ED50 values of 3-hydroxypyridin-4-ones (7) for the in-vitro inhibition of *P. falciparum*.

Hydroxypyridinone	R_1	R_2	ED50 (μM)
CP20	CH_3	CH_3	67
CP38	$CH_2CH_2CO_2H$	CH_3	56
CP110	$CH_2CH_2CO_2H$	CH_2 CH_3	53
CP31	CH_2CO_2H	CH_3	370
CP39	$CH_2CH_2CH_2CO_2H$	CH_3	250
CP85	$CH_2CH_2SO_3H$	CH_3	650

Although this value is much higher than those corresponding to conventional antimalarials, the anionic iron chelators (CP38 and CP110) are much less toxic to mammalian cells and levels of 50 μM are clinically attainable with oral administration of related hydroxypyridone CP20 (Kontoghiorghes et al 1990). Unfortunately, although CP20 possesses some antimalarial properties under in-vitro conditions, it lacks such activity when used clinically (Mabeza et al 1996) due to its extremely rapid metabolism (Singh et al 1992). CP38 and CP110, unlike CP20, are not glucuronidated by mammalian tissue.

Not surprisingly the very property that renders CP38 non-toxic to mammalian cells, leads to relatively poor oral absorption. In principle, this difficulty can be circumvented by the prodrug concept, utilising the ability of the liver to efficiently metabolize pyridinones (Hider et al 1996). A typical sequence of reactions which occurs in high yield in both rodents and man is depicted in Scheme 1. The prodrug ester is absorbed from the intestine with high efficiency. Molecules of this class are currently being investigated for their ability to function as antimalarials in in-vivo models.

References

Albert, A., Rees, C. W., Tomlinson, A. J. H. (1956) Why are metal binding substances anti-bacterial? Recueil 75: 819–824

Bunnag, D., Poltera, A. A., Viravan, C., Looareesuwan, S., Harinasuta, K. T., Schindlery, C. (1992) Plasmodicidal effect of desferrioxamine-β in human vivax or falciparum malaria from Thailand. Acta Trop. 52: 59–67

Cabantchik, Z. I. (1989) Altered membrane transport of malaria-infected erythrocytes: a possible pharmacological target. Blood 74: 1464–1471

Cabantchik, Z. I., Glickstein, H., Golenser, J., Loyevsky, M., Tsafack, A. (1996) Iron chelators: mode of action as antimalarials. Acta Haematol. 95: 70–77

Cooper, C. E., Lynagh, G. R., Hoyes, K. P., Hider, R. C., Cammack, R., Porter, J. B. (1996) The relationship of intracellular iron chelation to the inhibition and regeneration of human ribonucleotide reductase. J. Biol. Chem. 271: 20291–20299

Cory, J. G., Lasater, L., Sato, A. (1981) Effect of iron chelating agents on inhibitors of ribonucleotide reductase. Biochem. Pharmacol. 30: 979–984

Dobbin, P. S., Hider, R. C., Hall, A. D., Taylor, P. D., Sarpong, P., Porter, J. B., Xiao, G., Van Der Helm, D. (1993) Synthesis, physicochemical properties and biological evaluation of N-substituted 2-alkyl-3-hydroxy-4(1*H*)pyridinones: orally active iron chelators with clinical potential. J. Med. Chem. 36: 2448–2548

Elford, B. C., Cowan, G. M., Ferguson, J. P. (1996) Parasite-regulated membrane transport processes and metabolic control in malaria-infected erythrocytes. Biochem. J. 308: 361–374

Fritsch, G., Sawatzki, G., Treumer, J., Jung, A., Spira, D. R. (1987) *Plasmodium falciparum*: inhibition in vitro with lactoferrin, desferrithiocin and desferricrocin. Exp. Parasitol. 63: 1

Ganeshaguru, K., Hoffbrand, A. V., Grady, R. W., Cerami, A. (1980) Effect of iron chelating agents on DNA synthesis in human cells. Biochem. Pharmacol. 29: 1275–1279

Goldberg, D. E., Slater, A. F. G., Cerami, A., Henderson, G. B. (1990) Haemoglobin degradation in the malaria parasite *Plasmodium falciparum*: an ordered process in a unique organelle. Proc. Natl Acad. Sci. USA 87: 2931–2935

Golenser, J., Tsafack, A., Amichai, Y., Libman, J., Shanzer, A., Cabantchik, Z. I. (1995) Antimalarial action of hydroxamate-based iron chelators and potentiation of desferrioxamine action by reversed siderophores. Antimicrob. Agents Chemother. 39: 61–65

Gordeuk, V., Thuma, P., Brittenham, G., McLaren, C., Parry, D., Backenstrose, A., Biemba, G., Msiska, R., Holmes, L., McKinlye, E., Vargas, L., Gilkeson, R., Poltera, A. A. (1992a) Effect of iron chelation therapy of recovery from deep coma in children with cerebral malaria. N. Engl. J. Med. 237: 1473–1477

Gordeuk, Y. R., Thuma, P. E., Brittenham, G. M., Zulu, S., Simwanza, G., Mhang, A., Flesch, G., Parry, D. (1992b) Iron chelation with desferrioxamine β in adults with asymptomatic *Plasmodium falciparum* parasitemia. Blood 79: 308–312

Heppner, D. G., Hallaway, P. E., Kontoghiorghes, G. J., Eaton, J. W. (1988) Antimalarial properties of orally active iron chelators. Blood 72: 358–361

Hershko, C., Peto, T. E. A. (1988) Desferrioxamine inhibition of malaria is independent of host iron status. J. Exp. Med. 168: 375

Hershko, C., Gordeuk, V. R., Thuma, P. E., Theanacho, E. N., Spira, D. T., Hider, R. C., Peto, T. E. A., Brittenham, G. M. (1992) The antimalarial effect of iron chelator studies in animal models and in humans with mild falciparum malaria. J. Inorg. Biochem. 47: 267–277

Hider, R. C., Porter, J. B., Singh, S. (1994) The design of therapeutically useful iron chelators. In: Bergeron, R. J., Brittenham, G. M. (eds) The Design of Therapeutically Useful Iron Chelators in the Development of Iron Chelators for Clinical Use. CRC Press, Ann Arbor, pp 353–371

Hider, R. C., Choudhury, R., Rai, B. I., Dehkordi, L. S., Singh, S. (1996) Design of orally active iron chelators. Eur. J. Drug Metab. Disposit. 95: 6–12

Hoyes, K. P., Hider, R. C., Porter, J. B. (1992) Cell cycle synchronisation and growth inhibition by 3-hydroxypyridin-4-one iron chelators in leukamia cell lines. Cancer Res. 52: 4591–4599

Kannani, J., Ginsburg, H. (1991) Transport of lactate in *Plasmodium falciparum*-infected human erythrocytes. J. Cell Physiol. 149: 469–476

Kannani, J., Ginsburg, H. (1992) Effect of cinnamic acid derivatives on *in vitro* growth of *Plasmodium falciparum* and on the permeability of the membrane of malaria-infected erythrocytes. Antimicrob. Agents Chemother. 36: 1102–1108

Kontoghiorghes, G. J., Goddard, J. G., Bartlet, A. N., Sheppard, L. (1990) Pharmacokinetic studies in humans with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one. Clin. Pharmacol. Ther. 48: 255–261

Li, X.-M. (1986) Clinical analysis of the efficacy of daphnetin in the treatment of Bruger's disease in 112 cases. Jilin Med. 7: 28–29

Lytton, S. D., Mester, B., Dayan, I., Glickstein, H., Libman, J., Shanzer, A., Cabantchik, Z. I. (1993) Mode of action of iron(III) chelators as antimalarials: 1. Membrane permeation properties and cytotoxic activity. Blood 81: 214–221

Mabeza, G. F., Biemba, G., Gordeuk, V. R. (1996) Clinical studies of iron chelators in malaria. Acta Haematol. 95: 78–86

Meshnik, S. R., Yang, Y. Z., Lima, V., Kuypers, F., Kamchonwongpaisan, S., Yuthovong, Y. (1993) Iron-dependent free radical generation from the antimalarial agent artemisinin (Quinghasosu). Antimicrob. Agents Chemother. 37: 1108–1114

Nordlund, P., Sjoberg, B. M., Eklund, H. (1990) Three-dimensional structure of the free radical protein of ribonucleotide reductase. Nature 345: 593–598

Pasvol, G., Cough, B., Carlsson, J. (1992) Malaria and the red cell membrane. Blood Reviews 6: 183–192

Pattanapanyasat, K., Thaithong, S., Kyle, D. E., Udomsangpetch, R., Yongvanitchit, K., Hider, R. C., Webster, H. K. (1997) Flow cytometric assessment of hydroxypyridinone iron chelators on in vitro growth of drug-resistant malaria. Cytometry In press

Peto, T. E. A., Thompson, J. L. (1986) A reappraisal of the effects iron and desferrioxamine on the growth of *Plasmodium falciparum* in vitro: the unimportance of serum iron. Br. J. Haematol. 63: 273–280

Pollack, S., Rossan, R. N., Davidson, D. E., Escajadillo, A. (1987) Desferrioxamine suppresses *Plasmodium falciparum* in *Aotus* monkeys. Proc. Soc. Exp. Biol. Med. 184: 162–164

Porter, J. B. (1989) Oral iron chelators; prospects for future development. Eur. J. Haematol. 43: 271–285

Porter, J. B., Gyparaki, M., Burke, L. C., Huehns, E. R., Sarpong, P., Saez, V., Hider, R. C. (1988) Iron mobilization from hepatocyte monolayer cultures by chelators: the importance of membrane permeability and the iron-binding constant. Blood 72: 1497–1503

Porter, J. B., Morgan, J., Hoyes, K. P., Burke, L. C., Huehns, E. R., Hider, R. C. (1990) Relative efficacy and acute toxicity of hydroxypyridin-4-one iron chelators in mice. Blood 76: 2389–2396

Raventos-Suarez, C., Pollack, S., Nagal, R. L. (1982) *Plasmodium falciparum*: inhibition of in-vitro growth by deferoxamine. Am. J. Trop. Med. Hyg. 31: 919–922

Scheibel, L. W., Adler, A. (1980) Antimalarial activity of selected aromatic chelators. Mol. Pharmacol. 18: 320–325

Scheibel, L. W., Adler, A. (1982) III 8-Hydroxyquinolines (oxines) substituted in position 5 and 7 and oxines annelated in position 5, 6 by an aromatic ring. Mol. Pharmacol. 22: 140–144

Scott, M. D., Ranz, A., Kuypers, F. A., Lubin, B. H., Meshnick, S. R. (1990) Parasite uptake of desferrioxamine: a prerequisite for antimalarial activity. Br. J. Haematol. 75: 598–602

Shanzer, A., Libman, J., Lytton, S. D., Glickstein, H., Cabantchik, Z. I. (1991) Reversed siderophores act as antimalarial agents. Proc. Natl Acad. Sci. USA 88: 6585–6589

Singh, S., Epemolu, R. O., Dobbin, P. S., Tilbrook, G. S., Ellis, B. L., Damani, L. A., Hider, R. C. (1992) Urinary metabolic profiles in human and rat of 1,2-dimethyl- and 1,2-diethyl-substituted 3-hydroxypyridin-4-ones. Drug Metab. Dispos. 20: 256–261

Steck, E. (1971) The Chemotherapy of Protozoal Diseases. Walter Reed Army Institute of Research, Washington DC

Stubbe, J. (1990). Ribonucleotide reductases: amazing and confusing. J. Biol. Chem. 265: 5329–5332

Tsafack, A., Loyevsky, M., Ponka, P., Cabantchik, Z. I. (1996) Mode of action of iron (III) chelators as antimalarials. J. Lab. Clin. Med. 127: 574–582

Van Zyl, R. L., Havlik, I., Hemplemann, E., MacPhail, A. P., McNamaro, L. (1993) Malaria pigment and excellular iron. Possible target from iron chelating agents. Biochem. Pharmacol. 45: 1431–1436

Whitehead, S., Peto, T. E. A. (1990) Stage-dependent effect of desferrioxamine on growth of *Plasmodium falciparum* in vitro. Blood 76: 1250–1255

Yang, Y. Z., Ranz, A., Pan, H. Z., Zhang, Z. N., Lin, X. B., Meshnick, S. R. (1992) Daphnetin: a novel antimalarial agent with in vitro and in vivo activity. Am. J. Trop. Med. Hyg. 46: 15–20

Yinnan, A. M., Theanacho, E. N., Grady, R. W., Spira, D. T., Hershko, C. (1989) Antimalarial effect of HBED and other phenolic and catecholic iron chelators. Blood 74: 2166–2171