J. Pharm. Pharmacol. 1997, 49 (Suppl. 2): 49–53

© 1997 J. Pharm. Pharmacol.

Haem-mediated Decomposition of Artemisinin and its Derivatives: Pharmacological and Toxicological Considerations

GEOFFREY EDWARDS

Department of Pharmacology and Therapeutics, The University of Liverpool and Division of Parasite and Vector Biology, Liverpool School of Tropical Medicine

Resistance of *Plasmodium falciparum* to currently available antimalarial drugs is intensifying the search for novel, more effective chemotherapeutic agents. The most important discovery, isolated by Chinese scientists from *Artemisia annua*, is artemisinin (Klayman 1985; Meshnick et al 1996; Fig. 1). Despite impressive biological activity, problems with recrudescence and difficulties with formulation have led to the development of artemether and arteether. These are methyl and ethyl ethers of dihydroartemisinin, a lactone-reduced analogue of artemisinin and are more potent than artemisinin in-vitro and in-vivo in animal studies. Water soluble derivatives of dihydroartemisinin, artesunate and sodium artelinate have also been produced (Fig. 2). These analogues are at various stages of development. Artemether and sodium artesunate are in clinical use and are under evaluation in multicentre trials (Hien & White 1993), arteether is being developed jointly by the World Health Organization (WHO) and the Walter Reed Army Institute of Research and sodium artelinate is in phase I clinical trials (Olliaro & Trigg 1995). Clinical studies worldwide with artemisinin and its analogues have shown these drugs to be particularly effective in the treatment of severe malaria and in cases of *Plasmodium falciparum* infection unresponsive to treatment with existing antimalarial agents (White 1994; Li et al 1994; Hien 1994; Looareesuwan 1994; Salako et al 1994). Their efficacy and relatively low clinical toxicity has encouraged their widespread and occasionally unregulated use. A concern was raised, not unreasonably, that these drugs were being used against a background of pharmacological ignorance as, aside from the Chinese literature, there was relatively little definitive information on the pharmacology of these substances. Information was circulating within internal documents of the US Army and the WHO but not in peer-reviewed journals generally available to Western scientists. A major stimulus to activity in this area was provided by a meeting organized under the auspices of the Wellcome Trust in April 1993. At this meeting, a group of scientists and clinicians active in this field met to exchange views. While the success of artemisinin and its analogues was acknowledged, several gaps in our knowledge became apparent, notably the empirical nature of dosage regimens for these drugs and the evidence of neurotoxicity in preclinical studies.

Pharmacology

There was, until recently, relatively little published pharmacokinetic information on artemisinin in Western literature (Titulaer et al 1990) but a proliferation of activity in this area leads us to hope that rationally-derived dosage regimens might not be too far away (Duc et al 1994; Na Bangchang et al 1994; Hassan Alin et al 1996a, b). However, much of the available information is descriptive, and the relationship between pharmacokinetics and drug response is poorly understood. The principal drawback to acquiring such information is that the development of analytical methodology for measurement of

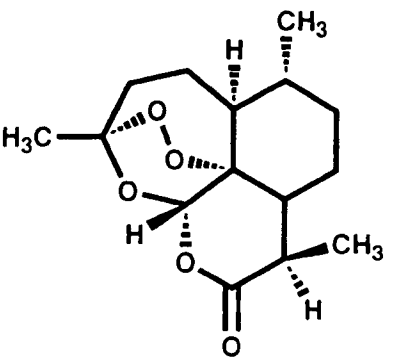

FIG. 1. Chemical structure of artemisinin.

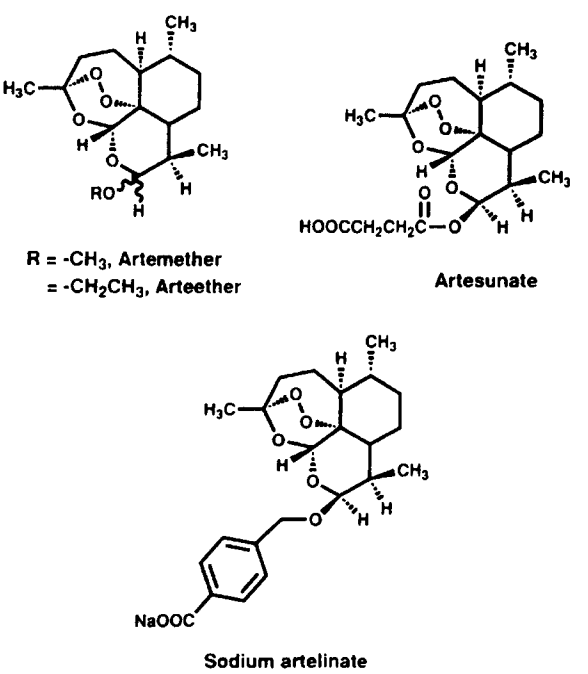

FIG. 2. Chemical structures of artemether, arteether, artesunate and sodium artelinate.

Correspondence: G. Edwards, Department of Pharmacology and Therapeutics, The University of Liverpool, Liverpool L69 3BX, UK. E-Mail: ge1000@liv.ac.uk

these agents in biological fluids poses challenging problems (Edwards 1994). There has also been concern about what should be measured and whether these measurements are accurate markers of biological activity (Edwards et al 1992). Two main approaches to analysis have been adopted. These have each used high-performance liquid chromatography (HPLC) with either electrochemical (EC) (Melendez et al 1991) or ultra-violet (UV) detection. None of the derivatives of artemisinin possesses a suitable chromophore, so pre- (Idowu et al 1989; Thomas et al 1992; Muhia et al 1994a or post-column (Edlund et al 1984; Batty et al 1996) derivatization has been used. While evaluating a method for the measurement of artemether in whole blood, it became clear that there were significant losses of this analyte in samples stored under different conditions. This suggests decomposition of artemether, sequestration within or binding to the erythrocytic membrane (Edwards et al 1992). Furthermore, work from Meshnick and co-workers pointed to the formation of an adduct of artemisinin and haem and ferriprotoporphyrin-catalysed decomposition of artemisinin and its analogues (Meshnick et al 1991; Hong et al 1994). Several lines of evidence point to the peroxide linkage, a common feature of the molecular structure of artemisinin and its analogues, playing a major role in these phenomena (Fig. 3). The chemistry of the haem-artemisinin interaction has been explored thoroughly by Posner and his colleagues (Cumming et al 1996) and will be reviewed in this issue. Our studies have demonstrated that two major products, probably hydroxylated desoxy derivatives, arise from the reaction of haemin in-vitro with radioactively labelled artemether. The rate and extent of formation of these species are time dependent and are enhanced by increasing the concentration of haemin. We have shown also that erythrocyte haemolysis products accelerate the decomposition of artemether (Muhia et al 1994b). In plasma samples from patients with malaria, breakdown products of haemoglobin may be present in sufficient quantities to facilitate the decomposition of artemisinin derivatives. All of this causes concern when attempting to relate drug concentrations to a pharmacological response or when building in blood clearance to a pharmacokinetic model. Since the stimulus for pharmacological effect is the unbound drug concentation, binding to blood cells should not pose a major difficulty. However, problems could arise if the rate of disappearance of artemisinin and its analogues from blood is relatively rapid compared with total blood clearance.

Neurotoxicity

There is some concern that the use of artemisinin and its analogues against a background of limited pharmacological information, despite wide clinical experience, could yield some unexpected observations. As if to emphasize this point, a series of in-vivo toxicity studies in the dog and rat, injected intramuscularly with artemether and arteether, revealed a dose-dependent neurotoxicity associated with movement disturbances and spasticity. The neuropathies were specific to the caudal brain stem and were characterized by swollen axonal processes and spheroid formation with associated axonal degredation and necrosis in myelinated axons (Brewer et al 1994a,b). Furthermore, studies in monkeys (*Macacca mulatta*) demonstrated a dose-dependent neuropathology in the same regions as rodent and canine brains (Petras et al 1994). These histological changes have been found in the absence of neurological signs or behavioural performance deficits (Genovese et al 1995). Recent observations in-vitro from this laboratory (Fishwick et al 1994) and the Walter Reed Army Institute of Research in the USA (Wesche et al 1994) have shown inhibition of neuronal cell proliferation. It may be argued that this is a manifestation of general cytotoxicity as other cell types, such as Ehrlich ascites tumour cells (Woerdenbag et al 1993) and 3T3 kidney fibroblasts have been shown to be susceptible to artemisinin, albeit at higher concentrations. It is therefore more relevant to examine in-vitro the effects of these agents under conditions more reflective of axonal growth and maintenance in-vivo. When cultured in medium lacking serum but containing dibutyryl cyclic AMP, Nb2a cells differentiate and project extensions or neurites that are branching varicose processes similar to those produced by sympathetic neurones in primary cell culture (Prasad & Hsie 1971). In addition, there is expression of a variety of neuronal proteins including growth-associated polypeptide GAP-43, the neurofilament triplet proteins and the enzyme ornithine decarboxylase (Abdulla & Campbell 1993). These neuronal properties are affected by a

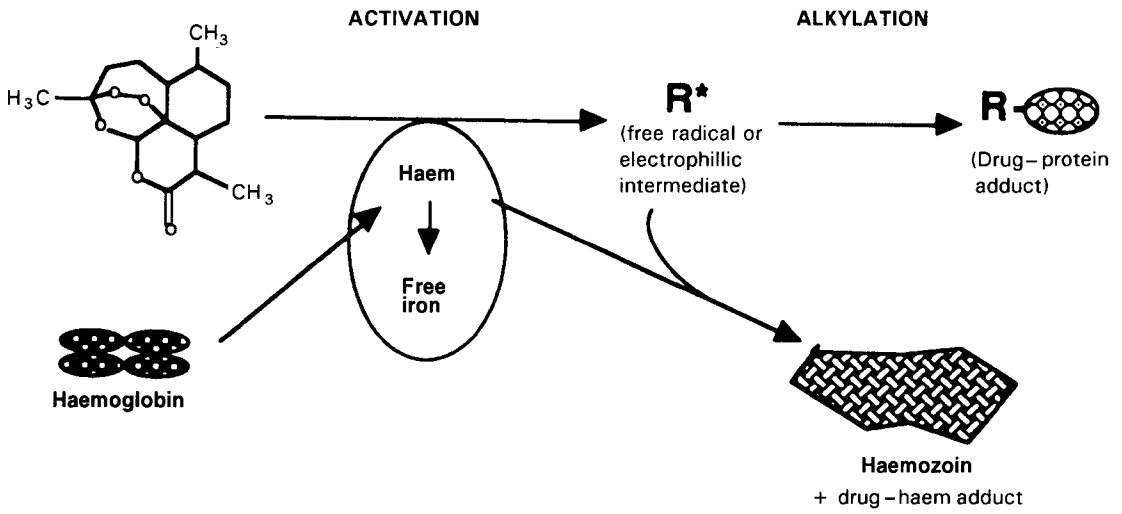

FIG. 3. Activation of artemisinin and its reaction with haem and proteins (from Meshnick 1994, with permission).

wide range of toxic agents and are subject to alteration in neuropathies. Therefore, Nb2a cells provide a good model for studying the effects of neurotoxins on nerve outgrowth in develoomment and regeneration, and also the integrity of the axonal cytoskeleton.

Looking at the effects of artemisinin, its derivatives and metabolites on the production of neurites in mouse neuroblastoma Nb2a cells, dihydroartemisinin was the most inhibitory. Recent findings from our laboratory have identified an IC50 value for dihydroartemisinin of approximately 0.35 μM. Other derivatives and analogues appear less toxic (Fishwick et al 1995). Such effects on neurite outgrowth occur at concentrations significantly less than those needed to reduce cellular proliferation and suggest some degree of specificity for neuronal cells. Proliferating neuroblastoma cells do not express the phenotypes of cells in the mature nervous system, for instance they do not possess axons or dendrites and effects on proliferation may not reflect selective neurotoxicity.

Clearly, if dihydroartemisinin crosses the blood–brain barrier to give concentrations in cerebrospinal fluid equivalent to those in serum (ca. 2.5 μM) then brain-stem toxicity might arise (Wesche et al 1994). However, much depends on the rate and route of administration and the relative conversion of the administered derivative to dihydroartemisinin as evidenced by the absence of reports of neurotoxicity in any clinical study (Phillips-Howard & ter Kuile 1995). Since these agents are used mostly in severely ill or comatose patients in whom there may be disease-related nerological sequelae, it may be impossible to identify drug-induced neurotoxicity in a clinical environment.

Enhancement of neurotoxicity by ferriprotoporphyrin

With a body of evidence pointing to a central role for ferriprotoporphyrin in the pharmacological actions of artemisinin, its derivatives and analogues, it is of interest to know whether their neurotoxicity is enhanced by haem. Using two methods of assessment, the metabolism of the tetrazolium salt 3-(4,5 diethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) or the inhibition of neurite outgrowth, haemin potentiates the neurotoxicity of artemether, arteether or dihydroartemisinin in a dose-dependent fashion without an effect by itself (Smith et al 1996) . Haemin also increases the concentration-related binding of radiolabelled (^{14}C) dihydroartemisinin to proteins from Nb2a cells two-fold and to rat brain three- to six-fold (Fishwick et al 1996). It has already been reported that antimarial endoperoxides react with specific proteins in the malarial parasite *Plasmodium falciparum* under physiological conditions (Asawamahasakda et al 1994) and that in haemoproteins, haem catalyses the alkylation of the protein moiety (Yang et al 1994). Haemin does not enhance the neurotoxicity of desoxyarteether, a structural analogue of arteether without an endoperoxide bridge (Smith et al 1996) and this analogue does not interact covalently with serum albumin (Yang et al 1993, 1994). These findings suggest the mechanism of neurotoxicity is similar to the mechanism of antimalarial activity. It is probable that haemin is catalysing the decomposition of artemisinin and its analogues to reactive species (Zhang et al 1992; Meshnick et al 1993) that are toxic to neuronal cells. Ferriprotoporphyrin (and free iron) can accelerate the decomposition of artemisinin and its derivatives through cleavage of the endoperoxide bridge to produce a carbon-centred radical (Posner & Oh 1992; Posner et al 1994, 1995a) possibly via an iron-oxo intermediate (Posner et al 1995b). It is unlikely that free iron, released from haemin during a reaction with artemisinin derivatives, is responsible for the neurotoxic effects as Fe^{2+} (ferrous sulphate) fails to enhance the toxicity of dihydroartemisinin in rat neurobastoma X glioma hybrid cells (Parker et al 1994). Damage may occur by lipid peroxidation or protein oxidation in neuronal membranes or cytoskeleton. Artesunate produces lipid peroxidation and oxidises thiol groups in isolated erythrocytic membranes (Meshnick et al 1989, 1991). Interestingly, one of the products of the iron-catalysed cleavage of endoperoxides like artemisinin, is a 1,5 diketone (Posner et al 1995a,b, 1996), a potent alkylating agent. This may crosslink cytoskeletal proteins in a fashion analogous to hexacarbon diketones such as 2,5 hexanedione (Genter St Clair et al 1988). Artemisinin binds to actin and, interestingly, the haem-containing protein spectrin, which is related closely to fodrin, a protein involved in cytosleletal maintenance (Asawamahasakda et al 1994; Lai & Singh 1995).

Conclusions

The isolation by Chinese scientists of artemisinin and the development of its derivatives can be considered to represent the most important breakthrough in malaria chemotherapy in recent times. Our increased understanding of the pharmacology of these agents allows the development of novel compounds of greater potency (Posner 1997). Moreover their unique mechanism of action should permit their use to continue with minimal interruption from the spectre of drug resistance that has dogged malaria chemotherapy with conventional chemotherapeutic agents. However, caution must be exercized and indiscriminate prophylactic usage could prove problematic. For severe malaria, it is vital that their use is rationalised and the role of sub-optimal therapy in the observed recrudescence be addressed. To do this, analytical methodology must be valid and appropriate. The question of whether preclinical neurotoxicity is an important clinical consideration remains uncertain, as no study has yet been designed to address this issue. Evidence from in-vitro investigations points to dihydroartemisinin, a common metabolite of artemether, arteether and artesunate, as being particularly neurotoxic, although rate and route of administration of the drug in question is important. Early evidence points to the half-life of dihydroartemisinin derived from intravenous artesunate being relatively short (Batty et al 1996). Alternatively, the benefit that these drugs undoubtedly convey in the treatment of severe disease may outweigh any theoretical risk of toxicity (Nosten & Price 1995). Importantly, neurological sequelae of cerebral malaria may not be distinguishable from drug-related effects. However, we should realise that we are dealing with chemicals that are organic peroxides of dubious stability and should be aware of unforeseen effects that might arise from their uncontrolled application.

Acknowledgements

Original work by the author was supported by the Medical Research Council, the Wellcome Trust and the UNDP/WHO/World Bank Special Programme for Research and Training in Tropical Diseases.

References

Abdulla, E. M., Campbell, I. C. (1993) Use of neurite outgrowth as an *in vitro* method of assessing neurotoxicity. Ann. NY Acad. Sci. 679: 276–279

Asawamahasakda, W., Benakis, A., Meshnick, S. R. (1994) The interaction of artemisinin with red cell membranes. J. Lab. Clin. Med. 123: 757–762

Batty, K. T., Davis, T. M. E., Thu, L. T. A., Binh, T. Q., Anh, T. K., Ilett, K. F. (1996) Selective high-performance liquid chromatographic determination of artesunate and α- and β-dihydroartemisinin in patients with falciparum malaria. J. Chromatogr. 677: 345–350

Brewer, T. G., Peggins, J. O., Grate, S. J., Petras, J. M., Levine, B. S., Weina, P. J., Swearengen, J., Heiffer, M. H., Schuster, B. G. (1994a) Neurotoxicity in animals due to arteether and artemether. Trans. R. Soc. Trop. Med. Hyg. 88 (Suppl. 1): 33–36

Brewer, T. G., Grate, S. J., Peggins, J. O., Weina, P. J., Petras, J. M., Levine, B. S., Heifer, M. H., Schuster, B. G. (1994b) Fatal neurotoxicity of arteether and artemether. Am. J. Trop. Med. Hyg. 51: 251–259

Cumming, J. R., Ploypradith, P., Posner, G. H. (1997) Antimalarial activity of artemisinin (qinghaosu) and related trioxanes: mechanism of action,. Adv. Pharmacol. 37: 253–297

Duc, D. D., De Vries, P. J., Khanh, N. X., Binh, L. N., Kager, P. A., van Boxrtel, C. J. (1994) The pharmacokinetics of a single dose of artemisinin in healthy Vietnamese subjects. Am. J. Trop. Med. Hyg. 51: 785–790

Edlund, P.O., Westerlund, D., Carlqvist, J., Bo-Liang, W., Yunhua, J. (1984) Determination of artesunate and dihydroartemisinine in plasma by liquid chromatography with post-column derivatisation and UV-detection. Acta. Pharm. Suec. 21: 223–234

Edwards, G. (1994) Measurement of artemisinin and its derivatives in biological fluids. Trans. R. Soc. Trop. Med. Hyg. 88 (Suppl. 1): 37–39

Edwards, G., Ward, S. A., Breckenridge, A. M. (1992) Interaction of arteether with the red blood cell *in vitro* and its possible importance in the interpretation of plasma concentrations in vivo. J. Pharm. Pharmacol. 44: 280–281

Fishwick, J., McLean, W. G., Edwards, G., Ward, S. A. (1995) The toxicity of artemisinin and related compounds on neuronal and glial cells in culture. Chem. Biol. Interact. 96: 263–271

Fishwick, J., McLean, W. G., Edwards, G., Ward, S. A. (1996) Investigation of the mechanism of neurotoxicity of the artemisinin derivatives. Trans. R. Soc. Trop. Med. Hyg. 90: 211

Genovese, R. F., Petras, J. M., Brewer, T. G. (1995) Arteether neurotoxicity in the absence of deficits in behavioural performance in rats. Ann. Trop. Med. Parasitol. 89: 447–449

Genter St Clair, M. B., Amaranth, V., Moody, A. M., Anthony, D. C., Anderson, C. W., Graham, D. C. (1988) Pyrrole oxidation and protein cross-linking as necessary steps in the development of γ-ketone neuropathy. Chem. Res. Toxicol. 1: 179–185

Hassan Alin, M., Ashton, M., Kihamia, C. M., Mtey, G. J. B., Bjorkman, A. (1996a) Multiple dose pharmacokinetics of oral artemisinin and comparison of its efficacy with that of oral artesunate in falciparum malaraia patients. Trans. R. Soc. Trop. Med. Hyg. 90: 61–65

Hassan Alin, M., Ashton, M., Kihamia, C. M., Mtey, G. J. B., Bjorkman, A. (1996b) Clinical efficacy and pharmacokinetics of artemisinin monotherapy and in combination with mefloquine in patients with falciparum malaria. Br. J. Clin. Pharmacol. 41: 587–592

Hien, T. T. (1994) An overview of the clinical use of artemisinin and its derivatives in the treatment of falciparum malaria in Vietnam. Trans. R. Soc. Trop. Med. Hyg. 88 (Suppl. S1): 7–8

Hien, T. T., White, N. J. (1993) Qinghaosu. Lancet 341: 603–608

Hong, Y-L., Yang, Y-Z., Meshnick, S. R. (1994) The interaction of artemisinin with malarial hemozoin. Mol. Biochem. Parasitol. 63: 121–128

Idowu, O. R., Edwards, G., Ward, S. A., Orme, M. L' E., Breckenridge, A. M. (1989) Determination of arteether in blood plasma by high-performance liquid chromatography with ultraviolet detection after hydrolysis with acid . J. Chromatogr. 493: 125–136

Klayman, D. L. (1985) Qinghaosu (artemisinin): an antimalarial drug from China. Science 228: 1049–1055

Lai, H., Singh, N. P. (1995) Selective cancer cell cytotoxicity from exposure to dihydroartemisinin and holotransferrin. Cancer Lett. 91: 41–46

Li, G-Q., Guo, X-B., Fu, L-C., Jian, H-X., Wang, X-H. (1994) Clinical trials on artemisinin and its derivatives in the treatment of malaria in China. Trans. R. Soc. Trop. Med. Hyg. 88 (Supppl. 1): 5–6

Looareesuwan, S. (1994) Overview of clinical studies on artemisinin derivatives in Thailand. Trans. R. Soc. Trop. Med Hyg. 88 (Suppl. 1): 9–11

Melendez, V., Peggins, J. O., Brewer, T. G., Theoharides, A. D. (1991) Determination of the antimalarial arteether and its deethylated metabolite dihydroartemisinin by high-performance liquid chromatography with electrochemical detection. J. Pharm. Sci. 80: 132–138

Meshnick, S. R., Tsang, T. W., Lin, F.-B., Pan, H.-Z., Chang, C.-N., Kuypers, F., Chiu, D., Lubin, B. (1989) Activated oxygen mediates the antimalarial activity of qinghaosu. Prog. Clin. Biol. Res. 313: 95–104

Meshnick, S. R., Thomas, A., Ranz, A., Xu, C.-M., Pan, H.-Z. (1991) Artemisinin (qinghaosu): the role of intracellular hemin in its mechanism of antimalarial action. Mol. Biochem. Parasitol. 49: 181–190

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

Meshnick, S. R. (1994) The mode of action of antimalarial endoperoxides. Trans. R. Soc. Trop. Med. Hyg. 88 (Suppl. 1): 31–32

Meshnick, S. R., Taylor, T. E., Kamchonwongpaisan, S. (1996) Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. Microbiol. Rev. 60: 301–305

Muhia, D. K., Mberu, E. K., Watkins, W. M. (1994a) Differential extraction of artemether and its metabolite dihydroartemisinin from plasma and determination by high-performance liquid chromatography. J Chromatogr. 660: 196–199

Muhia, D. K., Thomas, C. G., Ward, S. A., Edwards, G., Mberu, E. K., Watkins, W. M. (1994b) Ferriprotoporphyrin catalysed decomposition of artemether: analytical and pharmacological implications. Biochem. Pharmacol. 48: 889–895

Na Bangchang, K., Karbwang, J., Thomas, C. G., Thanavibul, A., Sukontason, K., Ward, S. A, Edwards, G. (1994) Pharmacokinetics of artemether after oral administration to healthy Thai males and patients with acute uncomplicated falciparum malaria. Br. J. Clin. Pharmacol. 37: 249–253

Nosten, F., Price, R. N. (1995) New antimalarials. A risk benefit analysis. Drug Safety 12: 264–273

Olliaro, P., Trigg, P. I. (1995) Status of antimalarial drugs under development. Bull. Wld. Hlth. Org. 73: 565–571

Parker, F. C., Wesche, D. L., Brewer, T.G. (1994) Does iron have a role in dihydroartemisinin-induced in vitro neurotoxicity? Am. J. Trop. Med. Hyg. 51: 260 (abstract)

Petras, J. M., Kyle, D. E., Ngampochjana, M., Young, G. D., Webster, H. K., Corcoran, K. D., Peggins, J. O., Brewer, T. G. (1994) Arteether-induced brainstem injury in *Macaca mulatta*. Am. J. Trop. Med. Hyg. 51: 100 (abstract)

Phillips-Howard, P. A., ter Kuile, F. (1995) CNS adverse events associated with antimalarial agents: fact or fiction? Drug Safety 13: 370–383

Posner, G. H., Oh, C. H. (1992) A regiospecifically oxygen-18 labelled 1,2,4-trioxane: a simple chemical model system to probe the mechanism(s) for the antimalarial activity of artemisinin (qinghaosu). J. Am. Chem. Soc. 114: 8328–8329

Posner, G.H., Oh, C. H., Wang, D., Gerena, L., Milhous, W. K., Meshnick, S. R., Asawamahasakda, W. (1994) Mechanism-based design, synthesis, and in vitro testing of new 4-methylated trioxanes structurally related to artemisinin: the importance of a carbon-centered radical for antimalarial activity. J. Med. Chem. 37: 1256–1258

Posner, G. H., Wang, D., Cumming, J. N., Oh, C. H., French, A. N., Bodley, A. L., Shapiro, T. A. (1995a) Further evidence supporting the importance of and the restrictions on a carbon-centered radical for high antimalarial activity of 1,2,4-trioxanes like artemisinin. J. Med. Chem. 38: 2273–2275

Posner, G. H., Cumming, J. N., Ploypradith, P., Oh, C. H. (1995b) Evidence for Fe(IV) = O in the molecular mechanism of action of

the trioxane antimalarial artemisinin. J. Am. Chem. Soc. 17: 5885–5886

Posner, G. H., Sheldon, B. P., Gonzalez, L., Wang, D., Cumming, J. N., Klinedinst, D., Shapiro, T. A., Bachi, M. D. (1996) Evidence for the importance of high valent Fe = O and of a diketone in the molecular mechanism of action of antimalarial trioxane analogs of artemisinin. J. Am. Chem. Soc. 118: 3537–3538

Posner, G. H. (1997) Antimalarial endoperoxides that are potent and easily synthesized. J. Pharm. Pharmacol. 49 (Suppl. 2): 55–57

Prasad, K. N., Hsie, A. W. (1971) Morphological differentiation of mouse neuroblastoma cells induced *in vitro* by dibutyryl adenosine 3'-5' cyclic monophosphate. Nature New Biol. 233: 141–142

Salako, L. A., Walker, O., Sowunmi, S. J., Omokhodion, S. J., Adio, R., Oduola, A. M. J. (1994) Artemether in moderately severe and cerebral malaria in Nigerian children. Trans. R. Soc. Trop. Med. Hyg. 88 (Suppl. 1): 13–15

Smith, S. L., McLean, W. G., Edwards, G., Ward, S. A. (1996) Haemin augements the in vitro neurotoxicity of artemisinin derivatives. Trans. R. Soc. Trop. Med. Hyg. 90: 215

Thomas, C. G., Ward, S. A., Edwards, G. (1992) The simultaneous determination, on plasma, of artemether and its major metabolite dihydroartemisinin by high-performance liquid chromatography with ultraviolet detection. J. Chromatogr. 583: 131–139

Titulaer, H. A. C., Zuidema, J., Kager, P. A., Wetseyn, J. C. F. M., Lugt, C. B., Merkus, F. W. H. M. (1990) The pharmacokinetics of artemisinin after oral, intramuscular and rectal administration to volunteers. J. Pharm. Pharmacol. 42: 810–813

Wesche, D. L., Decoster, M. A., Tortella, F. C., Brewer, T. G. (1994) Neurotoxicity of artemisinin analogs in-vitro. Antimicrob. Agents. Chemother. 38: 1813–1819

White, N. J. (1994) Artemisinin: current status. Trans. R. Soc. Trop. Med. Hyg. 88 (Suppl 1): 3–4

Woerdenbag, H. J., Moskal, T. A., Pras, N., Malingre, T. M. (1993) Cytotoxicity of artemisinin and related endoperoxides to Ehrlich ascites tumor cells. J. Nat. Prod. 56: 849–856

Yang, Y.-Z., Asawamahasakda, W., Meshnick, S. R. (1993) Alkylation of human albumin by the antimalarial artemisinin. Biochem. Pharmacol. 46: 336–339

Yang, Y.-Z., Little, B., Meshnick, S. (1994) Alkylation of proteins by artemisinin. Biochem. Pharmacol. 48: 569–573

Zhang, F., Gosser, D. K., Meshnick, S. R. (1992) Hemin-catalysed decomposition of artemisinin (qinghaosu). Biochem. Pharmacol. 43: 1805–1809