CONCENSUS ON THE USE OF ARTEMISININ DERIVATIVE CONTAINING COMBINATIONS (ADCC) FOR THE TREATMENT OF FALCIPARUM MALARIA

(Held in Cartagena, 2000).
XV Congress for Tropical Medicine and Malaria

Series Editors

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THE SOUTHEAST ASIAN JOURNAL OF TROPICAL MEDICINE AND PUBLIC HEALTH

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INTRODUCTION TO THE CONSENSUS MEETING

One of the problems with most conventional antimalarials is the presence of resistant clones within the parasite population which are rapidly selected and become the majority. Wallace Peters saw that using two drugs with different modes of action in combination would mean that the chance of resistance developing to both drugs would be very low. This would particularly be the case if the drugs potentiated each other's effects.

In 1987 his group reported that the arylcarbinolamine mefloquine, in combination with the endoperoxide artemisinin, showed potentiation against artemisinin-resistant rodent malaria in mice. Work in China then showed a potentiation in a combination of the arylcarbinolamine benflumetol (lumefantrine) and the endoperoxide artemether against mouse malaria and this was highly active against chloroquine-resistant *Plasmodium falciparum* in man (reported in a WHO symposium in 1989).

Recrudescence after monotherapy with endoperoxides is common in spite of their rapid effect and high kill rate. In the 1990s a rationale for the use of potentiating or non-potentiating companion drugs with endoperoxides in malaria therapy was developed by Nick White and collaborators. If a more slowly acting conventional drug were combined with the endoperoxide, White deduced that since the total parasite biomass was reduced to a very low level, it would be less likely to include clones which could resist the slower-acting drug.

Coartemether (Coartem®/Riamet®) is a combination of artemether and lumefantrine which could well have been designed to fulfil both Peters' and White's criteria, though in fact its development started well before White's views were promulgated.

In view of the WHO/TDR current encouragement of trials of endoperoxides in combination with all new antimalarials, the time is ripe for a discussion meeting on coartemether and its application to malaria control.

Important points needing to be discussed are the application of endoperoxides to reduce gametocyte load and hence transmission, and how to make coartemether available at low cost where it is really needed.

David Warhurst

MODE OF ACTION OF ARTEMETHER/LUMEFANTRINE (COARTEM®: THE SOLE, FIXED, ORAL ADDC) AND ITS ROLE IN COMBATTING MULTIDRUG-RESISTANCE

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INTRODUCTION

Lumefantrine binds to hemin produced during hemoglobin breakdown, preventing detoxification to crystalline malaria pigment (hemozoin). During the same process, the peroxide group in artemether binds to heme and releases toxic free-radicals. Resistance to both lumefantrine and artemether depends on expression of a multidrug-resistance protein PGH-1. This shared resistance mechanism is responsible for potentiation between the components of coartemether. Sensitivity to lumefantrine and artemether is determined by mutations in PGH-1 associated with chloroquine-resistance, but if mutated PGH-1 is over-expressed, resistance to all three drugs may be seen.

In Africa and South America, where PGH-1 is not generally found amplified, but chloroquine-resistance is widespread, resistance to either component of coartemether is expected to be rare. This feature should be maintained especially where chloroquine is still in regular use. In areas of Southeast Asia where chloroquine and mefloquine are widely used, both wild type and mutated PGH-1 are present and amplified. To combat the consequent low-level resistance to the combination, higher treatment doses of coartemether are required.

The advantage of coartemether as opposed to artesunate/mefloquine is seen in the shorter half-life of lumefantrine, allowing less time for resistance to be selected, and in coartemether's better tolerability. The selection of chloroquine-sensitivity determinants by coartemether, and of coartemether-sensitivity determinants by chloroquine suggests a possible strategy for resistance prevention in Africa and South America, which may be enhanced by probable effects of artemether on transmissibility of gametocytes.

LUMEFANTRINE

Lumefantrine is highly active against chloroquine-resistant malaria (Table 1), and has similarities to halofantrine and mefloquine. Like other arylaminocarbinol blood schizontocides (Chou *et al*, 1980; Warhurst, 1981), it binds strongly to ferriprotoporphyrin IX (hemin) produced during the breakdown of hemoglobin in the malaria parasite's digestive vacuole, preventing polymerization of the toxic iron porphyrin to non-toxic crystalline malaria pigment (hemozoin) (Bohle *et al*, 1997). The inhibition by lumefantrine can be seen *in vitro* (unpublished).

Resistance to lumefantrine

Resistance to mefloquine is associated experimentally and in field isolates (Price *et al*, 1999) with amplification of the *pfmdr1* gene specifying the multidrug resistance protein PGH-1. Conversely mutations in unamplified *pfmdr1* determine sensitivity to mefloquine and analogues (Reed *et al*, 2000). Recent
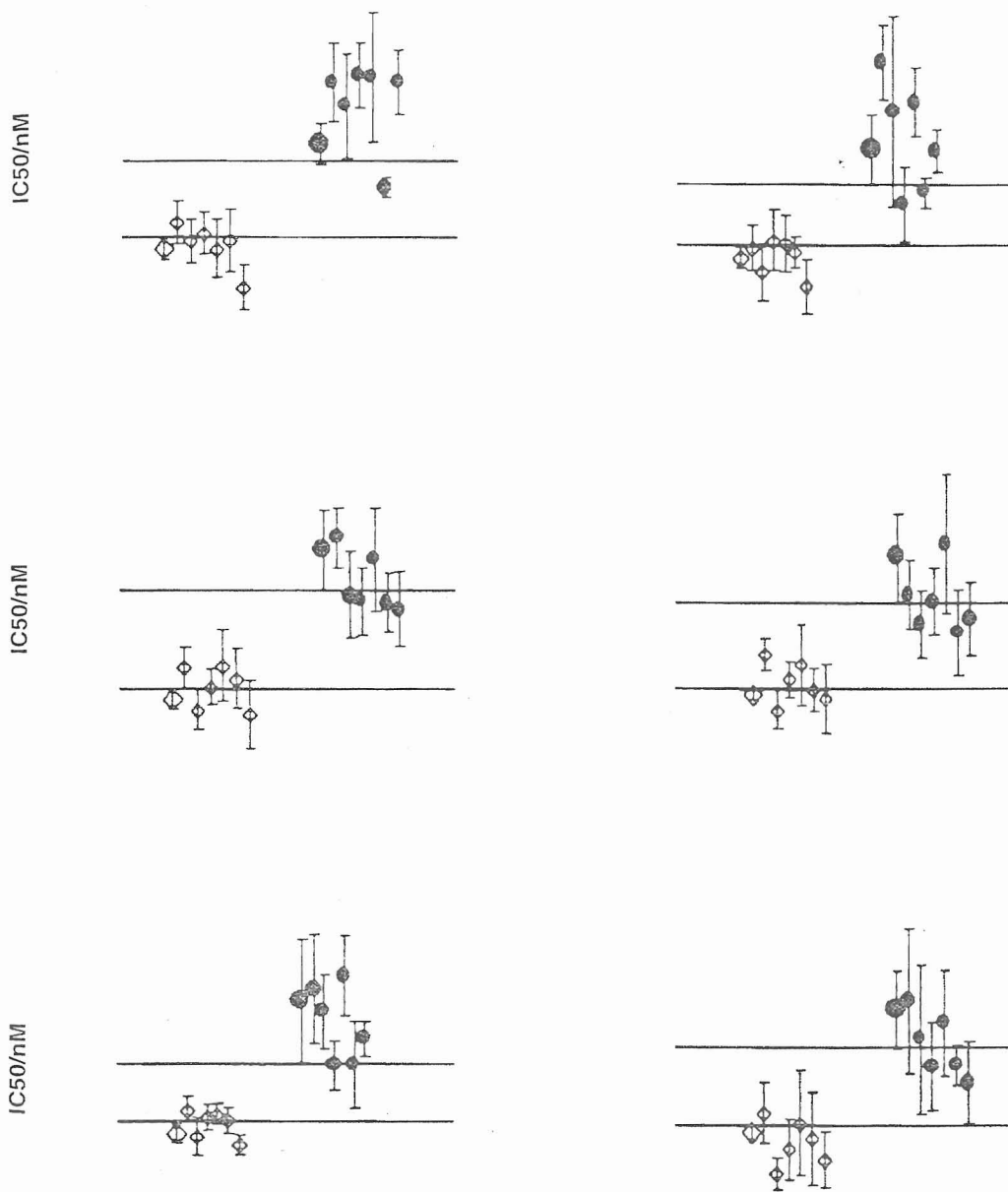
Fig 1

observations (Duraisingh *et al*, 2000) on an experimental cross between chloroquine sensitive clones 3D7 and HB3 (Table 1), both of which have single copies of *pfmdr1*, show that inheritance of sensitivity to lumefantrine, mefloquine and halofantrine in the progeny parallels inheritance of the HB3-like mutated *pfmdr1* allele (Fig 1).

Table 1

Genetic cross: Stage 1. Comparison of drug sensitivities between HB3 and 3D7 clones.				
Drug	IC50 HB3/nM (95% CI)	IC50 3D7/nM (95% CI)	p	IC50 3D7/HB3
Chloroquine	15.1 (12.7-17.5)	15.7 (14.6-16.8)	NS	1.03
Quinine	152 (110-194)	126 (124-129)	NS	0.83
Mefloquine	24 (21.9-26.1)	42.6 (39.6-45.6)	$<10^{-6}$	1.78
Halofantrine	5.14 (4.56-5.72)	10.8 (8.92-12.6)	0.001	2.09
Lumefantrine	34.6 (30.7-38.5)	87 (62-112)	0.001	2.51
Artermisinin	9.35 (8.5-10.2)	22 (18.5-25.5)	$<10^{-4}$	2.35
Dihydro-artemisinin	6.49 (1.93-11.1)	5.27 (2.46-8.08)	NS	0.81
Artemether	4.63 (4.44-4.82)	10.8 (8.8-12.8)	0.002	2.33
Arteflene	45.2 (42.2-48.2)	104 (85.5-123)	$<10^{-3}$	2.3
Atovaquone	0.97 (0.67-12.7)	1.33 (0.98-1.68)	NS	1.37

ARTEMETHER

The natural product artemisinin and derivatives are highly active blood schizontocides (Table 1) and reduce parasitemia rapidly *in vivo*. The peroxide group in artemether and other endoperoxides reacts with ferrous ion in ferroprotoporphyrin IX (heme) during the breakdown of hemoglobin in the malaria parasite's digestive vacuole, leading to the production of a carbon-centred-free-radical which inactivates nearby proteins by alkylation. Covalently-attached radiolabel can be detected after treatment, in a parasite homologue of a translation-controlled tumor protein (TCTP) of unknown function. Rodent malaria, *P. yoelii*, resistant to artemisinin, showed reduced dihydroartemisinin uptake, while also over-expressing wild-type TCTP (Walker *et al*, 2000).

It is currently believed that all artemisinin derivatives are metabolized to dihydroartemisinin which is responsible for the *in vivo* antiplasmodial effect. This may be true for artesunate, but there is no evidence that artemether and arteether, active agents *in vitro*, do not also act directly on the parasite *in vivo*. It is nevertheless important to remember that, because of the likelihood of metabolic transformations, *in vitro* observations on the endoperoxides may not necessarily apply *in vivo*.

RESISTANCE TO ARTEMETHER

There has been so far no clear evidence that resistance to endoperoxides, other than to arteflene (Radloff *et al*, 1996), is a clinical problem. It is sometimes stated that resistance to artemisinin derivatives cannot be detected *in vitro* in field or laboratory isolates, but a correct estimate of sensitivity depends on controlling for the inoculum effect. Properly controlled studies *in vitro* on field isolates, on an experimental cross (Fig 1), and after transfection, indicate that resistance is mediated by the same PGH-1 protein as for mefloquine and other similar drugs. PGH-1 may

act as a true multidrug resistance transporter, particularly effective in exporting hydrophobic drugs which dissolve well in membrane lipid. Where mutated PGH-1, associated with chloroquine-resistance (Adagu *et al*, 1996), is present, strains which are endoperoxide resistant show *pfmdr1* gene amplification, as in resistance to mefloquine. This amplification may ensure that sufficient of the defective transporter protein is present to remove the drug efficiently.

SELECTION OF RESISTANCE DETERMINANTS DURING TREATMENT WITH COARTEMETHER IN 2 FIELD TRIALS

In 1996 in the Gambia (von Seidlein *et al*, 1997) and in 1997 in Tanzania (Hatz *et al*, 1998), coartemether clinical trials were carried out in children. When parasite populations from day 0 of the Gambian trial were analysed (Duraisingh, 1999), it was found that 38.4% had *pfmdr1* tyr86 compared with 88% in the Tanzanian trial, reflecting the prevalence of chloroquine-resistance at the two sites. In the Gambian trial prevalence of *pfmdr1* tyr86 was significantly lower in recurring infections (day 14) after coartemether treatment, while in the Tanzanian trial, day 14 recurrences again showed significantly reduced prevalence of *pfmdr1* tyr86 and significantly increased prevalence of asn86. In a chloroquine treatment trial carried out as a control for the Tanzanian study, day 14 recurrences showed a significantly reduced prevalence of wild-type *pfmdr1* asn86.

ARYL-CARBINOLAMINE/ENDOPEROXIDE COMBINA-TIONS IN THE FACE OF RESISTANCE

The occurrence of potentiation between mefloquine/lumefantrine and artemisinin derivatives (Chawira *et al*. 1987; Hassan Alin

et al, 1999) gives a rationale for the use of combinations in addition to that proposed by White (1998). Potentiation is difficult to demonstrate experimentally because it is restricted to strains which have wild-type *pfmdr1* (showing some resistance to both drug types) (Duraisingh, 1999).

In areas like Africa and South America, where *pfmdr1* is not generally found amplified, but chloroquine-resistance is widespread, resistance to either component of coartemether is expected to be rare. This feature will be maintained especially where chloroquine is in regular use (Duraisingh, 1997).

In areas of Southeast Asia where use of chloroquine is infrequent and that of mefloquine is high, both wild type and mutated *pfmdr1* are present and amplified in the face of mefloquine pressure. The effect of the consequent low-level resistance to the combination can be detected in the need for higher doses of coartemether (van Vugt *et al*, 1998). The advantage of coartemether as opposed to artesunate/mefloquine is seen in the shorter half-life of lumefantrine, allowing less time for resistance to be selected, and in coartemether's better tolerability.

Reduction of production of mature game-tocytes has been noted following treatment with artemisinin derivatives and this has been associated in Thailand with a reduction in transmission (Price *et al*, 1996). Similar observations on gametocyte reduction have been reported for coartemether (von Seidlein *et al*, 1998).

USE OF COARTEMETHER AS PART OF A RESISTANCE-REDUCTION STRATEGY

The selection of chloroquine-sensitivity determinants by coartemether, and the selection of coartemether-sensitivity determinants by chloroquine suggests a sequential

strategy for avoiding resistance. It is also possible that use of artemisinin derivatives with chloroquine follow-up might encapsulate this strategy in a single treatment course.

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CONSENSUS STATEMENT

Following discussion, the following statement was agreed by the group:

Monotherapy of acute uncomplicated falciparum malaria may be unsuccessful and is susceptible to the development of resistance. Data from several areas support the use of drug combinations containing an artemisinin derivative.

The only fixed oral combination of this type currently available, Coartem® (artemether-lumefantrine) has been successfully tested in Africa and in areas of multidrug resistance in Southeast Asia, where its rapid action and good tolerability have been noted.

Coartem® is well-suited for first line treatment in multidrug resistant areas, and in other regions could be a useful second-line drug.