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MODERN MALARIA *

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Malaria is truly a disease of global significance and global impact. Forty per cent of the world's population still live in endemic areas and in addition, cheap mass air transportation facilitates importation of cases and re-introduction of the disease into non-endemic countries (Figure 1, see opposite). In 1993 three cases of indigenous falciparum malaria occurred in residents of Queens, New York, who did not have significant travel risk histories, who lived several miles upwind of air and sea ports and for whom the most likely source of infection was local mosquitoes which had acquired parasites from an unidentified imported case.[1]

The UK currently has one of the highest rates of imported malaria in the Western world (Figure 2); in 1996, 2,500 cases were recorded by the Malaria Reference Laboratory (personal communication) of which 1,283 were caused by *Plasmodium falciparum* (Pf).

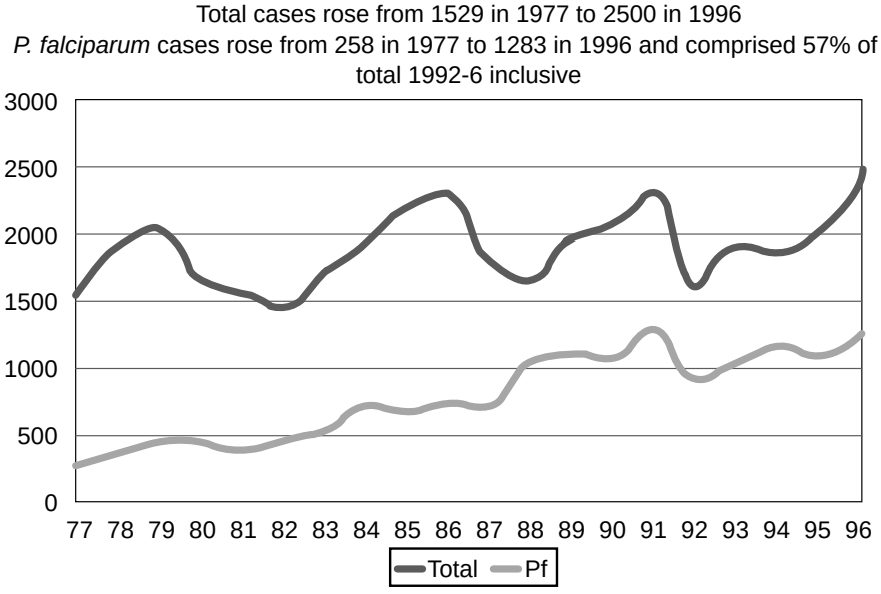

FIGURE 2
Malaria imported into the UK 1977-96.

Whilst the overall number of cases has risen and the proportion due to Pf has continued to increase, the number of deaths has remained remarkably constant, declining as

*Based on a paper presented at the Global Medicine Symposium at the Royal College of Physicians, Edinburgh on 24 October 1997.
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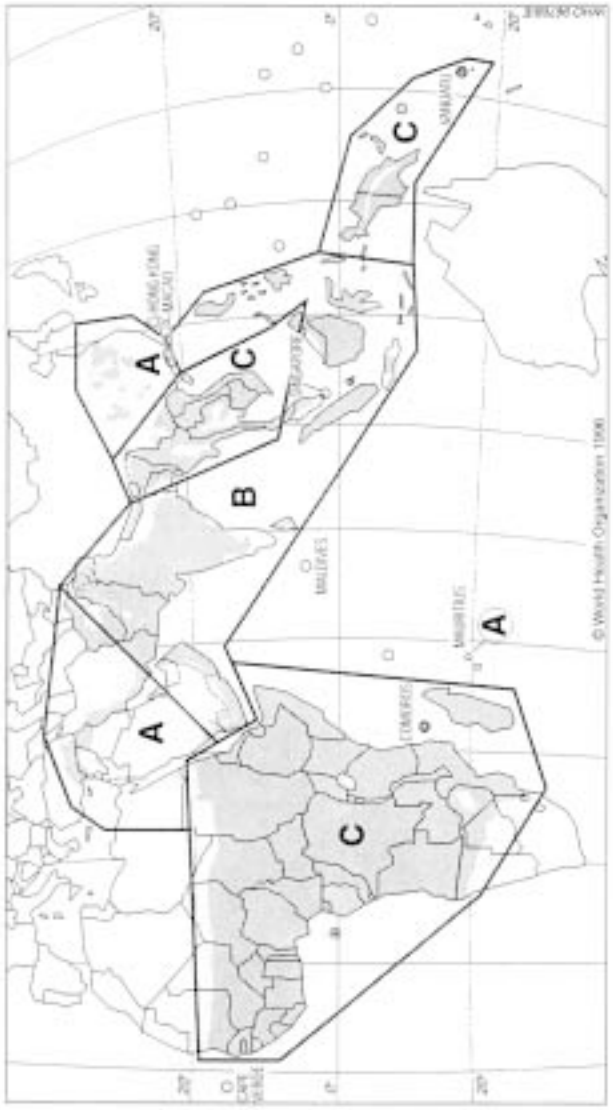

Zone	Characteristics			
A	In zone A, risk generally low and seasonal; no risk in many areas (for example urban areas) *P. falciparum* absent or sensitive to chloroquine.	*either:* *or:*	chloroquine prophylaxis (in case of very low risk) no prophylaxis	Protection from mosquito bites should be the rule in all situations, even when prophylaxis is taken
B	Low risk in most of the areas of zone B. Chloroquine alone will protect against *P vivax.* Chloroquine with proguanil will give some protection against *P falciparum* and may alleviate the disease if it occurs despite prophylaxis .	**prophylaxis:** *or:*	chloroquine + proguanil or /in case of very low risk) no prophylaxis	
C	In Africa, risk high in most areas of zone C, except in some high-altitude areas Risk low in most areas of this zone in Asia and America, but high in parts of the Amazon basin (colonization and minlng areas) Resistance to sulfadoxine pyrimethamine common in zone C in Asia, variable in zone C in Africa and America	**prophylaxis:** *or:*	prophylaxis: first choice mefloquine second choice - chloroquine + proguanil border areas Cambodia/Myanmar/Thailand - doxycycline or (in case of very low risk) no prophylaxis	

FIGURE 1
WHO map of malaria distribution. Reproduced with permission from *International Travel and Health Vaccination Requirements and Health Advice* WHO, 1997; 69.

a percentage of Pf cases from 1.9% for the years 1977-81 inclusive to 0.7% for the years 1992-6, suggesting more rapid diagnosis and/or commencement of treatment (Figure 3).

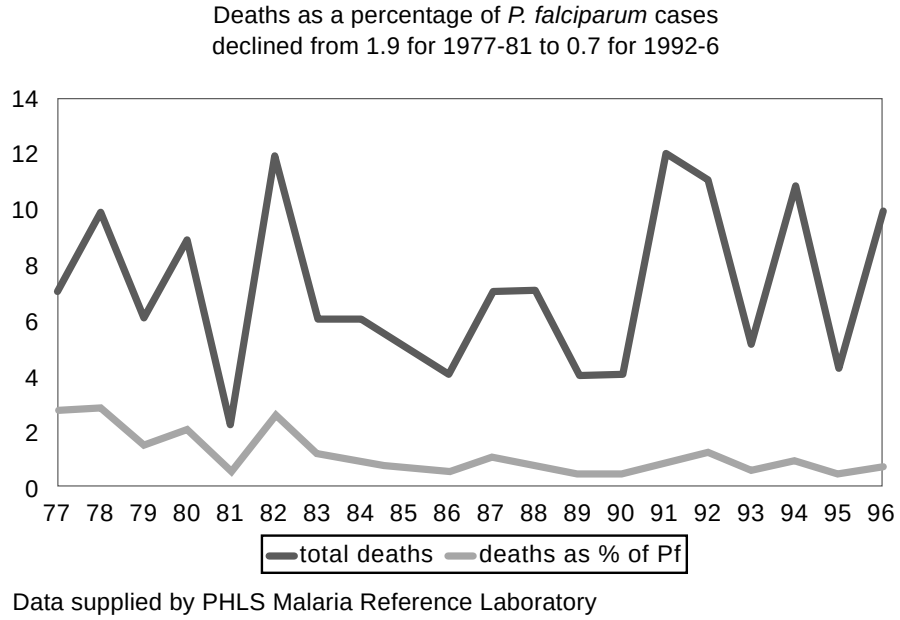

FIGURE 3
UK malaria deaths 1977-96.

Global malaria-related mortality and morbidity, as highlighted in the UNICEF Report *The Progress of the Nations*, remains enormous.[2] One million deaths occur annually in children under five years (one every 30 seconds) and more than half of all malaria episodes occur in children. Of the total of 400 million cases occurring worldwide, 90% occur in sub-Saharan Africa. The report also records some notable set-backs in the battle to reduce the impact of malaria. Water drainage and insecticide spray programmes have proved unsustainable in the worst areas; no vaccine is likely in the near future; most falciparum malaria is now resistant to chloroquine, the cheapest and most widely available medication; and resistance to second-line anti-malarials is increasing in south-east Asia and Brazil. The onward march of drug resistance has been inexorable over the last 50 years. The first reports for proguanil appeared in 1950, pyrimethamine in 1953, chloroquine in 1957, sulphadoxine-pyrimethamine (Fansidar) in 1967, mefloquine in 1983, quinine in 1987, and amodiaquine in 1988.[3] In addition, *P. vivax* shows increasing resistance to both chloroquine[4,5] and primaquine,[6] and some evidence is emerging that primaquine-resistance is also starting to have clinical impact.[7]

In the face of these major therapeutic and chemo-prophylactic setbacks, old agents have been used in new contexts and new agents are under trial. Advances have also been made in the application of transmission-blocking measures, notably bed nets, and less technologically demanding diagnostic methods.

BED NETS

The benefits of bed nets have been appreciated for many decades, and more recently

have been enhanced with impregnation with permethrin or other suitable insecticides. However, good scientific data supporting their use has only emerged more recently. Early studies have indicated a reduction in all-cause mortality by 25-70% among children aged 1-9 years, a 40% fall in hospital admissions for malaria, a 40% fall in children presenting with severe malaria, lowered rates and density of parasitaemia, lowered prevalence of anaemia and improved nutritional status.[8,9,10] A more recent study, however, failed to confirm an effect of bed nets on mortality or morbidity when controls were recruited from the same village as cases.[11]

Will any benefits prove durable, particularly in holoendemic areas? Semi-immunity to malaria is acquired only after about ten years of constant exposure, and is only maintained if that exposure is sustained. During infancy, maternal antibody gives waning protection, but malarial attacks start to induce active immunity during this early phase. One million deaths annually testify to the flaws in the adequacy of that immune response, but in general, in hyperendemic areas, high early childhood malaria mortality and morbidity give way to less severe disease as immune protection improves.[12] What will happen to childhood mortality and morbidity if attacks become less frequent due to the introduction of bed nets or other transmission-blocking measures?

Clinicians in Africa have observed anecdotally that intense perennial transmission seems to cause less mortality than intense seasonal transmission.[13] Since introducing bed nets will decrease the frequency of malaria exposure, it is therefore possible that they may decrease the degree of semi-immunity acquired by a population. Partial interruption of transmission could therefore spread the impact of severe and cerebral malaria throughout childhood or produce an overall increase in these severe complications. The period of observation in surveys on the impact of partial interruption of malaria transmission has mostly been short, 1-2 years, and direct evidence to support the hypothesis is lacking. However, a recent study by Robert Snow and colleagues[14] documents 'nature's experiment' as observed by Louis Molineaux, and reinforces these concerns.[13] Figure 4 summarises these data.

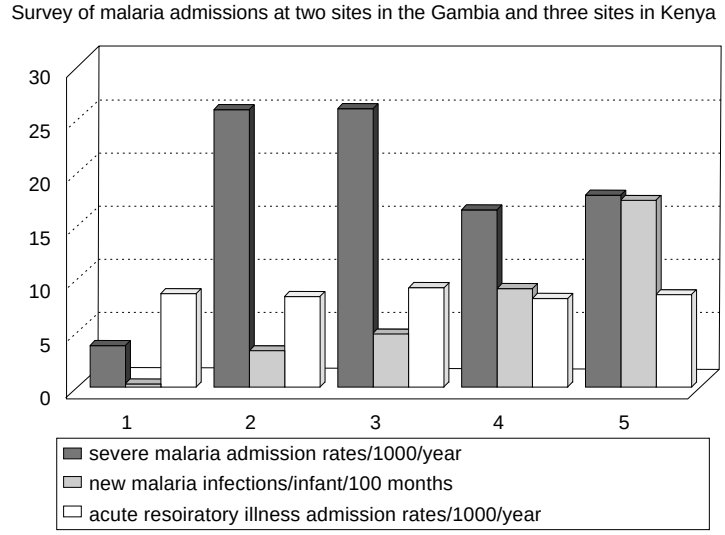

FIGURE 4
The risks of reducing malaria transmission. Reproduced with permission from Snow RW, Omumbo JA, Lowe B *et al*. Relation between severe malaria morbidity in children and level of *Plasmodium falciparum* transmission in Africa. *Lancet* 1997; 349:1650-4. © by The Lancet Ltd. [14]

Snow *et al.*[14] looked at five discrete communities in Kenya and the Gambia, recorded paediatric admissions with severe malaria over 3-5 years, and determined Pf malaria exposure by parasitological and serological surveys on children and infants. Acute respiratory illness (ARI) was used as a control illness in an attempt to identify whether there was selective use of hospitals by different communities and age groups, rendering these communities less comparable: ARI rates in the five communities were almost identical. Severe malaria occurred less frequently in communities with the highest rates of exposure to infection than in communities with intermediate exposure. This is made evident in Figure 4 by comparing the high rate of severe malaria in communities which had a low rate of new infantile malaria with 'Community 5', which had a much higher infantile new malaria rate, but less cases of severe malaria.

Snow's data do not justify withholding transmission-blocking measures for malaria, but do indicate that one needs to be alert to the possibility that severe lethal complications such as cerebral malaria may become more common.[14] As Molineaux observes, 'the jury is out' and we await the results of more research.[13] Transmission-blocking vaccines would have the same effect if the hypothesis holds, as would the widespread use of anti-malarial agents with gametocidal properties.

MALARIA VACCINES

Malaria vaccine research continues to have a roller-coaster ride. Several helpful reviews have appeared over the last few years,[15,16,17] and in this section I also refer to papers presented at the 14th Tropical Medicine and Malaria Symposium in Japan in late 1996 and the 5th International Conference on Travel Medicine held in Geneva in March 1997.

Sporozoite vaccines

The success of early vaccines, prepared from irradiated sporozoites, in protecting against infection prompted optimism even though they were not commercially viable.[16] The answer seemed to lie in identifying a reliably-expressed antigenic component of sporozoites: circumsporozoite protein (CSP) seemed ideal. However, CSP vaccines, while inducing antibody production, did not reliably prevent malaria.[16,18] It is now recognised that the success of radiation-attenuated sporozoite vaccines is due to their entry into hepatocytes stimulating a cytotoxic lymphocyte (CL) response to neutralise sporozoites.[17] Such enhanced sporozoite-directed vaccines may also kill infected liver cells, and impede emerging merozoites.

Several routes to improve on the initial CPS vaccines are being explored. Other malarial antigens may be added to CSP, or CSP may be fused with another protein known to stimulate CL responses; in this regard hepatitis B surface antigen (HBsAg) looks promising.[19] Alternatively CSP may be 'piggybacked' onto viruses and influenza,[16] adenovirus[20] and vaccinia[19 21] are potential candidates. Lastly DNA-based vaccines may also induce a CL response.

Merozoite vaccines

One vaccine (SPf66) has been subjected to extensive clinical trials over the last five years after encouraging preliminary results in Colombia. The vaccine incorporates three merozoite antigens and was developed by Manuel Patarroyo and colleagues.[22]

Four trials have now been performed (Figure 5): a Colombian study published in 1993[22] in adults and children, a trial in Tanzania published in 1994[23] in children aged 1-5 years, in the Gambia in 1995[24] in infants aged 6-11 months and in Thailand in 1996[25]

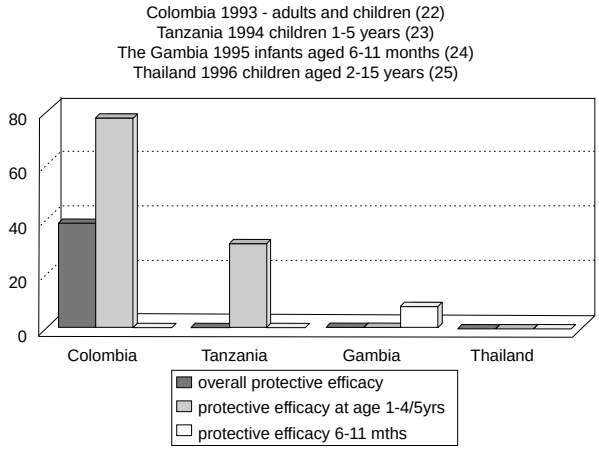

FIGURE 5
SPf66 (Patarroyo) vaccine trials.

in children aged 2-15 years. While benefit was demonstrated in Colombia, this was less marked in hyperendemic areas where such a vaccine might be expected to have its greatest application. Although these results are disappointing, they have not signalled the end of interest in merozoite vaccine research, and currently another merozoite vaccine utilising three antigens awaits efficacy trials in Papua New Guinea, Phase I initial safety trials having been successfully completed.[26]

Transmission-blocking vaccines

There are two potential antigen targets for vaccines at the departure point of the parasite from the human host, firstly antigens shared by gametocyte and gametes, and secondly ookinete antigens.[16] Neither will protect against infection of humans or subsequent disease, but both could block infectivity to mosquitoes. Ensuing natural infection will boost the effect of gametocyte-based vaccines but not ookinete vaccines.[16] Phase 1 human trials of the latter are imminent. Gametocyte-targeted vaccines have the potential of altering the pattern of severe malaria in childhood.

NEW DIAGNOSTIC METHODS

For 95 years the mainstay of laboratory diagnosis has been the thick blood smear. It takes 30-60 minutes of preparation, is labour-intensive and requires considerable experience for detecting low levels of parasitaemia.[27]

Acridine orange staining has been employed in some laboratories and was refined by Becton Dickinson into the quantitative buffy coat method (QBC).[26,27] A capillary tube coated with acridine orange is filled with blood and spun on a horizontal centrifuge. It is then viewed with paraviewer and fluorescent microscope. Whilst faster than preparing and examining a thick blood smear, it requires sophisticated and expensive equipment and speciation and estimating parasitaemia load is difficult.[28] In western hospital laboratories it has proved sensitive (99%) and specific (98%), but in tropical field conditions sensitivity falls to 70-79% although specificity is retained at 95-99%.[29]

Two relatively cheap alternatives however are now on the market which are likely to make a significant impact. Both are based on the detection of histidine-rich protein (HRP) which is produced in the knobs seen on the surface of infected erythrocytes. The ParaSight-F™ test uses a water soluble monoclonal antibody directed against

Pf HRP-2 deposited on a nitro-cellulose/glass fibre dipstick.[30] The Malaria Pf™ test uses two antibodies, one attached to visible colloidal gold on the absorbent strip and the other immobilised in a line across the test strip.[31] At present these methods only detect falciparum malaria, but are easy to learn: they may be very useful where there is no microscope, are quick to perform and provide accurate diagnosis in ten minutes. In field conditions the Malaria Pf™ test is preferred due to the shorter time it takes to obtain a result and the need for only one reagent.[32]

In field trials the ParaSight-F™ test achieved 93% sensitivity and 98% specificity in Thailand,[33] 90% sensitivity and 99% specificity in Sri Lanka[34] and 96.6% sensitivity for counts <100/uL in Burundi,[35] but takes up to 14 days to revert to negative after curative therapy.[32] False positive reaction has been reported in patients with rheumatoid factor.[36] The Malaria Pf™ test achieved 100% sensitivity, and 96.2% specificity in the Solomon Islands[30] and 100% sensitivity, 84.5% specificity in India with reversion to negative after 7 days.[37] Late apparent sero-reversion would otherwise indicate resistance and signal a change to alternative therapy if facilities for blood film examination were not available.

One study has helpfully compared the sensitivity of four methods: Giemsa thick film, acridine orange thick film, QBC and ParaSight-F™, and Figure 6 demonstrates the comparative sensitivities.[38] The 100% sensitivity cut-off measure is measured in parasites/mm^3 and the field sensitivities standard against 800 microscope fields of Giemsa thin film. Whilst field sensitivities are compared between the four methods, ParaSight-F™ appears to have an important advantage, detecting 100% of cases at much lower levels of parasitatria than the other three methods.

These methods have an important application in malaria endemic areas where there is no microscope or skilled technician, and non-endemic areas where technicians see rather few cases. This would include cities like Edinburgh where 20-30 cases of malaria are diagnosed every year.

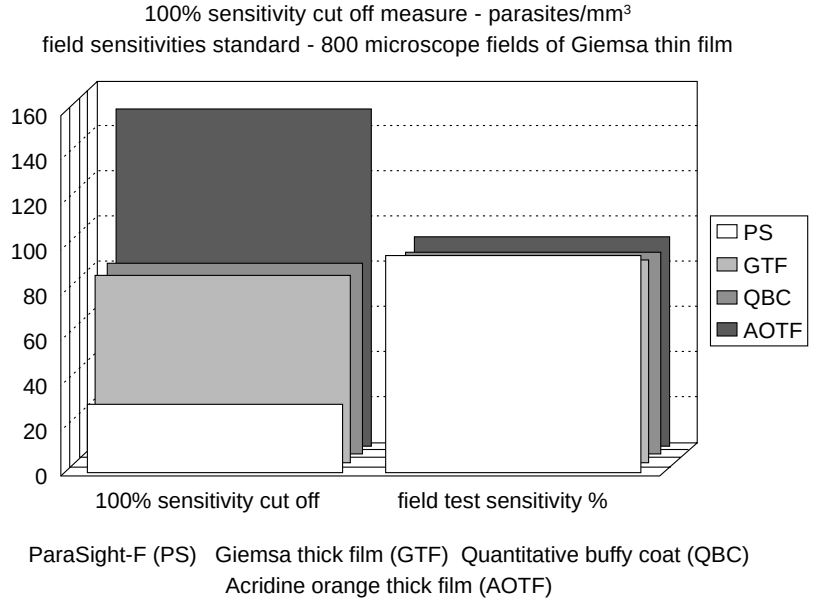

FIGURE 6
Evaluation of four diagnostic techniques for malaria.[38]

PROPHYLAXIS AND TREATMENT

Mefloquine

Patricia Schlagenhauf has observed 'While the effectiveness of mefloquine is widely accepted its tolerability remains questionable....A major problem has been to identify just exactly what is being measured in the various studies....Globally-accepted definitions are rarely applied'.[39]

The issue of side-effects has recently received particular attention in the British media. In Switzerland, when mefloquine is prescribed for travellers they rarely question it (Steffen R, personal communication), whilst in the UK many travellers view the possibility of using mefloquine with considerable anxiety.

A number of studies have attempted to identify the level of side-effects and here I review four of them plus a recent meta-analysis. The first major prospective study examined outcomes in 1,322 Peace Corps Volunteers working in West Africa between 1989 and 1992.[40] Volunteers were monitored monthly by medical officers for suspected adverse effects (AE), a four-monthly volunteer questionnaire was completed on compliance and AE, plus monitoring of mefloquine levels during latter part of study. Mild adverse events were equally frequent with chloroquine plus proguanil (C + P) and mefloquine (M). No serious adverse effects were observed.

The massive malaria prophylaxis study mounted by Steffen and colleagues drew similar conclusions.[41] The study was conducted in two parts, Malpro-1 (1985-8) and Malpro-2 (1988-91), and comprised a questionnaire study of 145,003 travellers returning to Europe on flights from Mombasa in Kenya. Malpro-1 was more specific about cutaneous reactions and Malpro-2 about neuropsychiatric reactions. Side-effects were more frequent with C + P (30%) than with M (19%), and there were few serious neuropsychiatric effects, 1/13,600 for C + P, and 1/10,600 for M.

Croft and World published the results of a prospective double-blind trial in 624 fit British male military personnel randomised to C + P or M.[42] No overall significant difference in adverse effects was detected and no serious neuropsychiatric reactions were seen. Intermediate neuropsychiatric effects were almost identical at 41-42%. The first serious neuropsychiatric adverse effect was witnessed after the study finished in 1995: 'a 63 kg 26-year-old private experienced a visual hallucination of "the grim reaper" standing behind the chaplain and auditory hallucination of incoherent voices'.[43] In 1995 an Australian study reported on 285 travellers on mefloquine and 383 on doxycycline, enrolled pre-travel and mailed two weeks after return (Figure 7).[44] Significant adverse effects were twice as common in females (15%) on mefloquine than males (6%), and strange dreams were reported in 10% compared to only 2% on doxycycline. The high rate of significant adverse effects in females taking doxycycline (9%) compared to males (5%) reflects vaginal thrush. Despite the high rate of adverse effects experienced in those using mefloquine, compliance with mefloquine was better than doxycycline, probably due to the advantage of weekly over daily dosing.

The most recent questionnaire-based retrospective study analysed 2,397 travellers with equal numbers taking C + P and M.[45] Travellers were contacted on return with reminders at 4 and 8 weeks, and had equal rates of stopping or changing prophylactic. Total side-effects were equal at 40% but neuropsychiatric events (NPAE) occurred in 27% on M and 16% on C + P. These were disabling in 0.8% (10) for M and 0.09% (1) for C + P. Eight out of ten of these disabling NPAE on mefloquine occurred in females. Although there was an excess of hospital admission for 2:1214 on M and 1:1181 on C + P, this was not statistically significant. The authors concluded that there was a significant excess of intermediate neuropsychiatric events for those on mefloquine.

285 travellers on mefloquine and 383 on doxycycline, enrolled pre-travel and mailed 2 weeks after return

	female	complete compliance	significant adverse effects in females	significant adverse effects in males	strange dreams
mefloquine	60%	78%	15%	6%	10%
doxycycline	48%	68%	9%	5%	2%

FIGURE 7
Australian questionnaire study - adverse side-effects.

Re-examination of the Malpro-2 data suggests that older travellers are less likely to have side-effects (7% in those >59 years) than younger travellers (16% in those 20-59 years).[64] Meta-analysis identified ten trials carried out in non-immune travellers where an attempt had been made to conduct a randomised comparison of mefloquine against placebo or against alternative standard prophylaxis.[47] The authors concluded that whilst mefloquine had been shown in one study to be effective, withdrawal rates, presumed due to side-effects were high across most studies, and this may reduce the usefulness of mefloquine for routine prophylaxis.

In conclusion, whilst mefloquine is not as safe as originally hoped, it appears very much safer than was implied by the BBC *Watchdog* programme. A more definitive answer awaits the completion of a Canadian prospective multicentre trial which at March 1997 had reached a study population size of over 500 subjects.[48] Although studies of motor performance have not shown any problems, mefloquine should not be prescribed for airline pilots, women in the first trimester of pregnancy, infants or those with a personal or strong FH of serious mental ill health or epilepsy. It seems wise to avoid alcohol and recreational drugs whilst taking mefloquine.

Other therapeutic developments

Five years ago the future seemed bleak with the prospect of untreatable malaria at the end of the century. Concern still remains high but new agents and fresh combinations of existing agents are being tried.

Primaquine has been used for radical cure of vivax malaria (Pv) for several decades. More recently it has been under trial as a prophylactic for both Pf and Pv. Placebo-controlled trials have been conducted for one year in Javanese in Irian Jaya[49] and in Kenyan children.[50] The dose of 0.5 mg/kg, (30 mg daily for adults) gave protective efficacy of 95% in Irian Jaya and 79% in Kenya. The action of primaquine against liver-stage parasite gives it a causal prophylactic capability and for this reason may only need to be taken for 2-3 days after leaving endemic areas.[46]

Etaquine, formerly known as WR 238605, has a long half-life of 2-4 weeks which makes it particularly suitable as a prophylactic agent for which once weekly dosing has major advantages if high compliance is to be achieved. It kills all stages of the parasite, has an anti-gametocidal effect, and entered field trials as prophylactic in 1997.[51]

Pyronaridine was originally synthesised in 1971, in China. It has both anti-schizonticidal and significant anti-gametocidal activity greater than that of primaquine, giving it major transmission blocking potential.[52] It is effective in a three-day treatment regimen at 32mg/kg for three days and in Cameroon gave 100% cure compared to 44% for chloroquine.[53] It causes less pruritus but more gastrointestinal side-effects than chloroquine and its toxicological profile is not yet established. Thus far few patients have been treated with it outside China and the drug has many hurdles to clear before its place and safety are established.[54]

Qinghaosu is the original Chinese name given to extracts of the sweet wormwood plant *Artemisia annua*, sometimes used as a flavouring for vermouth.[55] Six compounds have now been synthesised from this extract: artemesinin, dihydro-artemesinin, artemether, artesunate, arteether and artelinate. All these have a rapid onset of action, a short half-life, appear to have anti-gametocidal action, are easily hydrolysed, and are unstable in aqueous solution.[56] Toxicity appears low and fears about neurotoxicity have not been confirmed clinically: in cerebral malaria artemether is as effective as quinine.[57] Arteether is now close to being licensed in Holland as Articef™ and is stable in oil solution for five years.

Early recrudescence has been a problem when these agents have been used on their own, and the expert view is that any qinghaosu derivatives must be combined with another anti-malarial, and the combination of artesunate and mefloquine is highly effective in Thailand.[58] One such combination is CGP 56697 (artemether + benflumetol), transiently named co-artemether. Benflumetol is a class 2 aryl amino alcohol with a similar site of action to quinine. The combination is given in four doses in fixed dose schedule over 24 hours. Cure rates are in the region of 94-98% at 28 days and UK license application was scheduled for late 1997.[59,60,61]

The activity of the combination of atovaquone plus proguanil (Malarone™) is related to the interaction between the drugs and two metabolites cycloguanil, and p-chlorophenyl-biguanide, making it a four drug potentiating combination.[62] On the Thai-Myanmar border it gave a 100% cure rate in MDR cases,[63] 99% in Brazil,[64] 87% in Gabon,[65] and 94% in Kenya.[66] One confirmed RII level resistance recrudescence was seen in the Kenyan treatment trial. It has considerable prophylactic potential and a probable causal prophylactic action since re-infection is prevented beyond five serum half lives.[67] It was granted a license in UK in 1996 as second/third-line oral malaria treatment.

CONCLUSION

Malaria remains, in research terms, a comparatively impoverished Cinderella. Graham Brown, Head of Infectious Diseases at the Institute of Medical Research in Melbourne, noted recently that total global expenditure on malaria research is only $84 million compared to $900 million for HIV.[68] Global expenditure per fatal case is $42 for malaria in contrast to almost $800 for asthma; this at a time when global warming encourages the spread of malaria to higher altitudes and more extreme latitudes, threatening the life and health of greater numbers of indigenous people and travellers. The malaria researchers whom I have met are men and women of calibre and commitment. That commitment in the context of a disease which has an enormous impact on tropical health deserves a larger slice of research funding than it is currently allocated and such a re-apportioning of funding could potentially save the lives of millions.

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