

unrelenting itching. No longer will people be threatened by gross deformity from filariasis. Irrespective of personal views on the merits or achievability of malaria eradication, it is the confidence in the availability of fast acting and highly effective first, second or third generation ACTs that has contributed to the debate to ensure we are in a position where we do have the tools to attempt the audacious challenge of regional elimination. Eradication is, at present, an aspirational goal without powerful new tools and needs to be placed in the context of the definition of eradication – ‘global zero incidence of a specific organism’. Nevertheless, in the absence of the work of Youyou Tu and her team at Mission 523, it is probable that malaria would currently be a disease on the rise and one where treatment options were severely, life-threateningly limited. Millions of lives have been saved by the availability of ACTs and as cases fall [10] and we move away from the hideous statistic of more than 1 million lives, mainly children, lost per year to this treatable infection, we enter a future where a world without the disease can be contemplated with significant economic benefits for endemic countries [11]. Whatever the future, we are much better placed to treat malaria now than where we were before the availability of ACTs. In reflecting on this Nobel Prize, it is clear that many parallels can be drawn between the discovery, development, deployment and eventual impact of ivermectin and artemisinin. The malaria and helminth scientific and public health communities did not interface and, instead, developed independently. However, to achieve public health impact, common approaches were needed – public–private partnerships, global advocacy, NGDO involvement, subsidised pricing mechanisms or drug donations. Such processes define the complex path from scientific discovery, through to global health policy change, implementation, monitoring and evaluation to ensure the poorest communities benefit from

access to critical medicines. Whilst much remains to be done and many challenges remain, the Nobel Committee has made an enlightened decision – to recognize the science that has led to making the lives of millions of the poorest significantly better than it was 50 years ago. In the next decades, with malaria and NTDs now embedded within the United Nations Sustainable Development Goals, the drugs developed by Campbell, Ōmura and Youyou Tu will be critical to achieve the targets for these diseases established by the international development community – some drugs, some legacy!

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References

1. Campbell, W.C. (1992) The genesis of antiparasitic drug ivermectin. In *Inventive Minds* (Weber, R.J. and Perkins, D. N., eds), pp. 194–214, Oxford University Press
2. Aziz, M.A. *et al.* (1982) Efficacy and tolerance of ivermectin in human onchocerciasis. *Lancet* 2, 1456–1457
3. Campbell, W.C. (1991) Ivermectin as an antiparasitic agent for use in humans. *Annu. Rev. Microbiol.* 45, 445–474
4. Awadzi, K. (1986) The chemotherapy of onchocerciasis XI: a double-blind comparative study of ivermectin, diethylcarbamazine and placebo in human onchocerciasis in northern Ghana. *Ann. Trop. Med. Parasitol.* 80, 433–444
5. Liese, B. *et al.* (2010) Programmes, partnerships, and governance for elimination and control of neglected tropical diseases. *Lancet* 375, 67–76
6. Molyneux, D.H. (2014) Neglected tropical diseases: now more than just ‘other diseases’ – the post-2015 agenda. *Int. Health* 6, 172–180
7. Cross, C. *et al.* (2015) Historical profiles and perspectives from river blindness to neglected tropical diseases – lessons learned in Africa for programme implementation and expansion by the non-governmental partners. *PLoS Negl. Trop. Dis.* 9, e0003506
8. Klayman, D.L. (1985) Qinghaosu (Artemisinin): an antimalarial drug from China. *Science* 228, 1049–1055
9. Makanga, M. (2014) A review of the effects of artemether-lumefantrine on gametocyte carriage and disease transmission. *Malar. J.* 13, 291
10. World Health Organization (2014) *World Malaria Report*, WHO
11. Sachs, J. and Malaney, P. (2002) The economic and social burden of malaria. *Nature* 415, 680–685

Science & Society A Brief History of Qinghaosu

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The 2015 Nobel Prize for Medicine or Physiology was awarded to William C. Campbell and Satoshi Ōmura for their discovery of avermectins, and to Tu You You for her contribution to the discovery of artemisinin. The discovery and development of qinghaosu (artemisinin) as an antimalarial drug is a remarkable and convoluted tale.

In the mid-1960s, in the immediate aftermath of the Cultural Revolution, China responded to requests from North Vietnam for help in their impending conflict. Malaria had played a major role in the first and second World Wars, and it had nearly killed Ho Chi Minh in 1945. Ho knew malaria could be a decisive factor in the forthcoming struggle. Malaria was still a significant problem in China too and so scientists across the land were ordered to find effective remedies, both in modern pharmaceutical chemistry and also in the extensive traditional medicine pharmacopoeia. On the 23 of May 1967, Project 523 was formed. This remarkable truly multicentre collaboration discovered the antimalarial properties of organic extracts of the leaves of *Artemisia annua*, a traditional febrifuge, identified the antimalarial moieties, determined their chemical structures, and characterized their physico-chemical properties and their antimalarial activities, first in animal models, and then in human malaria [1]. Initially there was some confusion over the plant; was it qinghao or huanghuahao that had the magical antimalarial properties?

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ANTIMALARIA STUDIES ON QINGHAOSU

*Qinghaosu Antimalaria Coordinating Research Group**

An effective antimalaria constituent was extracted from a traditional Chinese medicinal herb — Qinghao (*Artemisia annua* L) 青蒿 in 1972. It was named Qinghaosu 青蒿素. According to the data from spectral analysis, X-ray diffraction analysis and chemical reaction it is a new type sesquiterpene lactone with a peroxy-group. Pharmacologic studies and clinical observations in every type of malarial infection show that Qinghaosu is a new type antimalaria drug with rapid action and low toxicity. It has direct parasitocidal action on plasmodium in the erythrocytic stage. The parasites in *plasmodium vivax* and *plasmodium falciparum* (including cerebral malaria and chloroquine-resistant *falciparum* malaria) especially in the areas of chloroquine-resistant *falciparum* malaria were cleared more rapidly than that with chloroquine and quinine etc, it is ineffective in the tissue stage. In general the short term recurrence rate is higher with Qinghaosu than with chloroquine.

of new types of antimalaria drugs then became important to antimalaria research. There is rich experience in antimalaria work in traditional Chinese medicine and pharmacology and a new antimalaria drug, Qinghaosu was extracted from a traditional Chinese herb.

The medicinal herb Qinghao 青蒿 (*Artemisia annua* L.) has been used in China for about 2,000 years. It was first described in "52 Prescriptions" unearthed from the Mawangdui Han Dynasty Tomb.¹ It was also recorded in "Shennong Bencao Jing"² published in the 1-2 century AD. The treatment of malaria with Qinghao was recorded in "Zhouhou Bei Ji Fang"³ in 341 AD, the handbook of prescriptions for emergencies. It says, take a handful of Qinghao.

Trends in Parasitology

Figure 1. The Remarkable First Publication in English Describing the Discovery and Development of Qinghaosu.

Misidentification delayed proceedings but once the correct plant extract was used, it was clear that the active moiety (now called qinghaosu, or later—artemisinin) was an extremely active antimalarial. Indeed it produced the most rapid parasite clearance of any known antimalarial drugs. Skillful chemistry then produced the reduction derivative dihydroqinghaosu (dihydroartemisinin) which was even more potent, and this served as the basis for stable lipophilic and hydrophilic derivatives (artemether and artesunate, respectively). Led by Professor Li Guo Qiao, a professor of traditional Chinese medicine from Guangzhou, clinical trials were conducted which confirmed the extraordinary antimalarial activity of these compounds both in uncomplicated and cerebral malaria [2–4]. In 1979 the Qinghaosu Antimalaria Coordinating Research Group published a remarkable succinct description (in English)

of the physicochemical properties, antimalarial activity, and clinical evaluation of artemisinin in the *Chinese Medical Journal* [2] (Figure 1). Slowly, the news spread.

Thereafter things went neither smoothly or quickly. The Chinese interaction with TDR, the World Health Organization (WHO) Special Programme for Research and Training in Tropical Diseases, which had a strong US Army representation at the time, was uneasy and ultimately fruitless. With dubious scientific justification, TDR decided to develop the ethyl ether (arteether) of dihydroartemisinin as a new drug, rather than the methyl ether (artemether) produced by a Chinese pharmaceutical company (the Chinese had actually synthesized arteether but rejected it in favour of artemether). Questions were raised over the quality of the Chinese drugs, and the stability of the water soluble artesunate. WHO TDR and

the US Army decided initially to focus only on the intramuscular oil based injections and not to develop oral or intravenous drugs, which today form the mainstay of antimalarial treatment (http://apps.who.int/iris/bitstream/10665/61147/1/TDR_CHEMAL_ART_86.3.pdf). Meanwhile, antimalarial drug resistance continued to worsen in Southeast Asia. Effective alternatives treatments were needed desperately. Asian investigators, tired of waiting for the 'quality' products promised by WHO, began studies with Chinese oral, parenteral and rectal formulations in Myanmar, Vietnam (which by the late 1980s was producing its own artemisinin), Thailand [5–7] and soon after in Africa [8]. These rapidly confirmed the original Chinese claims.

The first large randomized controlled trials in severe malaria, which started in 1991,

were with Chinese artemether [9]. These showed superiority in terms of mortality reduction in adults from Southeast Asia, but not in African children and left sufficient equipoise that quinine (another venerable plant derived compound) remained as the treatment of choice [10]. The oral drugs (artemisinin, artesunate, or artemether) were rapidly effective and well tolerated but as monotherapies they required treatment courses of 5 and 7 days in vivax and falciparum malaria, respectively. Combinations with more slowly eliminated antimalarial drugs proved highly effective and, eventually, 3-day treatments became established [4,6,7]. The excellent tolerability and efficacy of the artemisinin-based combination therapies (ACTs) in Southeast Asia eventually led to confirmatory trials in South America and across Africa, which began in the late 1990s [7]. Although the artemisinin derivatives were very well tolerated as well as being rapidly effective there were two prevailing safety concerns at the time; first, repeated high injected doses of the oil based arteether (and artemether) caused an unusual pattern of selective neurotoxicity affecting certain brain stem nuclei in rodents and beagle dogs; second, artemisinins were embryotoxic. Fortunately, neurotoxicity was never confirmed in humans, but until recently artemisinins were contraindicated in the first trimester of pregnancy in uncomplicated malaria infections, although there is increasing evidence for safety in early pregnancy.

During the 1990s it became increasingly clear that the continued use of inexpensive, yet ineffective, antimalarial drugs (chloroquine and then sulphadoxine-pyrimethamine) by most malaria endemic countries was killing millions of people (most of whom were children in Africa). Meanwhile, the evidence that ACTs were highly effective and well tolerated had grown steadily. The consistently good results from Asia were replicated elsewhere and toxicity concerns receded [7–10]. The debate between malaria researchers, non-governmental organizations

(NGOs), and growing numbers of malaria control programme representatives who argued strongly for deployment of these drugs, and the donors and international organisations who were reluctant to support them, became increasingly heated [11]. Finally in 2006, 27 years after the first seminal publication in English (Figure 1), the WHO decided clearly and unequivocally to recommend ACTs as first line treatment of uncomplicated falciparum malaria in all endemic countries [12]. At the same time, the WHO raised the bar substantially in the minimum efficacy required of an antimalarial treatment – malaria control programmes everywhere were now requested to aim for 28-day ‘cure’ rates of 95% and to change policy if cure rates fell below 90% [12]. Previously it had been considered acceptable for failure rates assessed at 14 days to be as high as 25% (which corresponded to true failure rates over 50%).

The rapid parasite clearance caused by the artemisinins and the associated speedy clinical recovery had long suggested that these compounds conferred a survival benefit in severe malaria. Unfortunately, because the oil-based intramuscular formulations (artemether, arteether) were then the compounds favoured by the WHO, these were the first to be evaluated in large randomized trials in severe malaria. The results were not sufficiently powerful to change practice [10], probably because these oil based intramuscular drugs are slowly and unreliably absorbed from the injection site. In contrast, the water-soluble artesunate can be given intravenously and is rapidly and reliably absorbed following intramuscular injection. Belatedly in 2003 multicentre randomized trials with parenteral artesunate began in Asia. These showed a substantial (35%) reduction in mortality compared with quinine [13]. This result was sufficient for policy change outside Africa, and it paved the way for the largest randomized controlled trial in African children hospitalised with severe malaria (AQUAMAT) [14]. The AQUAMAT trial showed a 22.5%

lower mortality in children who received artesunate compared with those who received quinine. This coincided with removal of lingering concerns over drug quality and led to a uniform recommendation for parenteral artesunate as the treatment of choice for severe malaria everywhere.

In recent years substantial increases in international funding for malaria control have resulted in widespread deployment of ACTs in nearly all malaria endemic areas, and contributed to a substantial decline in global malaria morbidity and mortality (World Malaria Report 2014: http://www.who.int/malaria/publications/world_malaria_report_2014/en). Malaria elimination is again on the political agenda. Although there are formidable obstacles to this ambitious goal, it cannot be achieved without effective antimalarial medicines. In January 2006, the WHO recognized the risks of artemisinin resistance arising from decades of poorly regulated use and widespread availability of falsified and sub-standard medicines, and pushed strongly for a ban on monotherapies, but unfortunately, this was too late to prevent the emergence of resistance to artemisinin. Today artemisinin resistant *Plasmodium falciparum* can be found across Southeast Asia from the coast of Vietnam to the Myanmar–India border [15]. Predictably, uncontained resistance to artemisinin has led to worsening resistance to the ACT partner drugs. The prospect of drug resistant malaria parasites spreading from Southeast Asia through India to Africa and killing millions of children for a third time has rightly excited alarm, and provoked numerous meetings and resolutions, but it has not resulted in a radical containment strategy. For most of the malaria affected world, there is no evidence yet that the products of this remarkable Chinese traditional medicine are failing – but continued vigilance is needed. Loss of the artemisinins would deal a devastating blow to our renewed ambitions to eliminate malaria.

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References

- Zhang, J.F. *et al.*, (2003) *A Detailed Chronological Record of Project 523 and the Discovery and Development of Qinghaosu (Artemisinin)*, (translated by K. and M. Arnold) Strategic Book Publishing, <http://sbpra.com/zhangjianfang>
- Qinghaosu Antimalaria Coordinating Research Group (1979) Antimalaria studies on Qinghaosu. *Chin. Med. J. (Engl.)* 92, 811–816
- Li, G.Q. *et al.* (1982) Clinical studies on treatment of cerebral malaria with qinghaosu and its derivatives. *J. Tradit. Chin. Med.* 2, 125–130
- Jiang, J.B. *et al.* (1982) Antimalarial activity of mefloquine and qinghaosu. *Lancet* 2, 285–288
- Myint, P.T. and Shwe, T. (1986) The efficacy of artemether (qinghaosu) in *Plasmodium falciparum* and *P. vivax* in Burma. *Southeast Asian J. Trop. Med. Public Health* 17, 19–22
- Nosten, F. *et al.* (1994) Treatment of multi-drug resistant *Plasmodium falciparum* malaria with 3-day artesunate-mefloquine combination. *J. Infect. Dis.* 170, 971–977
- Adjuk, M. *et al.* (2004) International Artemisinin Study Group. Artesunate combinations for treatment of malaria: meta-analysis. *Lancet* 363, 9–17
- White, N.J. *et al.* (1992) Comparison of artemether and chloroquine for severe malaria in Gambian children. *Lancet* 339, 317–321
- Tran, T.H. *et al.* (1996) A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. *N. Engl. J. Med.* 335, 76–83
- The Artemether–Quinine Meta-analysis Study Group (2001) A meta-analysis using individual patient data of trials comparing artemether with quinine in the treatment of severe falciparum malaria. *Trans. R. Soc. Trop. Med. Hyg.* 95, 637–650
- Attaran, A. *et al.* (2004) WHO, the Global Fund, and medical malpractice in malaria treatment. *Lancet* 363, 237–240
- World Health Organisation (2006) *Guidelines for the Treatment of Malaria*, WHO
- Dondorp, A. *et al.* (2005) Artesunate versus quinine for treatment of severe falciparum malaria: a randomised trial. *Lancet* 366, 717–725
- Dondorp, A.M. *et al.* (2010) Artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT): an open-label, randomised trial. *Lancet* 376, 1647–1657
- Tun, K.M. *et al.* (2015) Spread of artemisinin-resistant *Plasmodium falciparum* in Myanmar: a cross-sectional survey of the K13 molecular marker. *Lancet Infect. Dis.* 15, 415–421

Spotlight

A Bradyzoite is a Bradyzoite is a Bradyzoite?

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Bradyzoite forms of *Toxoplasma gondii* persist in tissue cysts for the lifetime of an infected host and can reactivate to cause clinical disease. It was thought that *in vivo* bradyzoites within tissue cysts are biologically inactive dormant forms that rarely replicate. Apparently, consensus was wrong.

The opportunistic pathogen *Toxoplasma gondii* is an Apicomplexan parasite that has the unique ability to persist within its host as latent bradyzoite forms that lie within tissue cysts. The rapidly growing tachyzoite form that is responsible for clinical disease is controlled by the immune system and differentiates into bradyzoites. Bradyzoites have unique antigens and metabolism that are presumed to protect them from the immune response and facilitate long-term viability in tissue [1] (Figure 1). Bradyzoites are important because they can reactivate to cause lethal disease in an immunocompromised host. Although *T. gondii* can infect all nucleated cells, cysts are common in the brain, and clinical toxoplasmosis often presents as encephalitis, acute inflammation of the brain. Bradyzoites are also infectious when ingested. So the biology of bradyzoites has been the subject of intense interest to scientists investigating pathogenesis of toxoplasmosis.

Recently, Watts *et al.* took a closer look at the dynamics of *T. gondii* bradyzoites within tissue cysts [2]. After painstakingly optimizing the classic purification protocol developed by Cornelissen [3], Watts *et al.* examined the size, density, and

bradyzoite contents of cysts harvested at different times from mouse brains. Ninety-nine cyst preparations, 630 cysts, and two years later, they found that more goes on within tissue cysts than had been appreciated previously.

Bradyzoite biology has been difficult to study. In the laboratory, bradyzoite differentiation can be induced by various environmental stresses, but it is difficult to obtain pure populations of bradyzoites that are not contaminated with tachyzoite forms. Strains of *T. gondii* differ in their propensity to differentiate, and strains cultivated in the laboratory gradually lose their ability to differentiate. Epigenetic gene regulation also plays an important role in the tachyzoite-bradyzoite transition, but the exact molecular triggers for differentiation are not understood, as it has not been possible to follow the progression of bradyzoite differentiation over time. There has been controversy in the field whether laboratory induced bradyzoites are ‘real’ bradyzoites, with bradyzoites harvested from tissue cysts serving as the gold standard reference. It has been assumed that *in vivo* bradyzoites are homogeneous and static. The careful, standardized quantitation and characterization of cysts by Watts *et al.* [2] reveals unexpected heterogeneity amongst bradyzoites and tissue cysts.

Taking advantage of advances in three-dimensional digital confocal imaging, the authors developed the BradyCount software that enabled them to accurately and efficiently determine bradyzoite number within cysts. In general, as expected, as cyst size increased, the numbers of bradyzoites within cysts increased. They also carefully measured cyst size and used digital imaging approaches to quantify parasite division within cysts.

There was a surprisingly amount of variability in cyst density and bradyzoite burden within individual cysts, suggesting