The Royal Society of Medicine

Journal of the Royal Society of Medicine; 2017, Vol. 110(1) 31–40
DOI: 10.1177/0141076816681421

Evaluating *Cinchona* bark and quinine for treating and preventing malaria

G Gachelin, P Garner, E Ferroni, U Tröhler and I Chalmers
Laboratoire SPHere, UMR 7219, CNRS-Université Paris 7, 75205 Paris, Cedex 13, France
Corresponding author: G Gachelin. Email: ggachel@club-internet.fr

This is the first part of this article and the other half will be published in the next issue, February 2017.

Part 1: Evaluation of *Cinchona* bark in the 17th and 18th centuries

We know a lot about malaria: the epidemiology of the disease and the genome of the parasites that infect us. We have made great advances, with new and effective drugs, methods to control the vectors, rapid diagnostic tests and the potential for effective vaccines. We have seen malaria gradually disappear from temperate climates by the 1950s. Despite all these advances, the disease still remains a cause of widespread poor health in many tropical areas.

Although we know malaria causes fever, to this day defining when a fever is caused by malaria remains a challenge. The clinical signs of fevers, including those caused by malaria, have been known for centuries, if not millennia (see Aulus Cornelius Celsus[1] as an example). From the beginning of the 18th century, however, malaria gradually emerged – from at least 128 different fevers recorded between 1774 and 1794 by Vicq d'Azyr (see Peter)[2] – as a distinct clinical entity within the still complex group of intermittent (periodic) fevers.[3]

During the 18th century, albeit with some continuing ambiguity,[4] malaria gradually became accepted as a defined set of intermittent fevers responding to 'therapeutic tests' using *Cinchona* bark or, from the 1820s, by using quinine. But difficulties in the diagnosis of genuine malaria persisted until the causal parasite and vectors had been recognised at the end of the 19th century.

After examining clinical and statistical data on malaria gathered during the 19th century, the Malaria Commission of the League of Nations concluded that few of the data were sufficiently reliable to be used in comparative studies.[5] The principal writer of the report, Nicolaas Swellengrebel,[6] pointed out that a shift in the meaning of the word malaria had occurred: from 'mal-aria' (bad air) as the cause of many fevers, to the name of a disease (plasmodiosis) reflecting the causative parasites – *Plasmodia*.

Comparison with past clinical experience and the rapidity of the response to both *Cinchona* bark and quinine in many reported cases and case series left comparatively little room for doubt that the drug had beneficial effects on the disease. As noted at the end of the 19th century by a German writer promoting careful statistical evaluation of treatments in general, 'to substantiate the efficacy of . . . quinine in malaria, one may not need statistics'.[7]

By contrast, it was not possible to make similarly confident causal inferences about the effects of most other interventions, whether purported advances in treating malaria or measures to control and prevent malaria at the population level. Multiple interventions were often poorly characterised and delivered without any formal comparison groups. Uncertainties and disputes resulted from this lack of formal comparative studies, with inevitable confusion about which antimalarial policies to apply, particularly at the population level.[5,8]

From the end of the 19th century, when the life cycle and vector of malarial parasites were identified, other antimalarial interventions were deployed beyond drugs, including, for example, approaches to limit breeding places for mosquitoes; provide physical protection of buildings and people against the insects; and to deploy antilarval and antimosquito procedures, such as petrol, larvivorous fish and Paris Green as a pesticide.

In this paper, we consider methods used from the 17th to the 21st centuries to assess the effects of *Cinchona* bark and of quinine and its derivatives for (i) treating and (ii) preventing malaria in individuals and (iii) in attempts to control malaria in populations.

The introduction of Cinchona *bark to Europe during the 17th century*

From its first documented use by the Spanish in Peru around 1630, the history of *Cinchona* bark is a

mixture of facts and legends, first compiled by Baldi as early as 1663,[9] and subsequently researched by numerous authors.[10–14] Although the bark was not included in the Inca pharmacopeia, it appears to have been used by Andean populations to combat shivering. The medicine became known, among other names, as *Cortex peruanus*, or Jesuit's powder, since it had been imported into Europe from Latin America by the Loyola Order. During the second half of the 17th century, the bark was used increasingly to treat fevers – and intermittent fevers in particular, which were shown 200 years later to be caused by malarial parasites.

The *Schedula Romana*, published in 1649, is an early example of an efficient antimalaria recipe, generally assumed to have been designed by the Spanish cardinal Juan de Lugo and to have summarised trials that he probably carried out.[10] The doses recommended are likely to have been established by trial and error, and it can reasonably be assumed that de Lugo relied on results obtained using the various recipes proposed by Roman apothecaries. In brief, two drachms (7.5 to 9 g) of selected bark (no details specified) were to be ground to a very fine powder (a special mill was established in Rome) and infused in hot, strong wine. This mixture was to be administered every day (sometimes several times a day). A rough computation based on the probable content in quinine of red bark leads to the conclusion that doses used in 1650 were in the range of those used after the isolation of quinine, that is, between 0.75 and 1.5 g of quinine a day.

The market became flooded with *Cinchona* barks of various efficacy, as well as with ineffective and even poisonous, barks from other trees, such as holly. In 1640, the efficacy of different parts of the tree, as well as the role of growth conditions in their content of the unknown 'active principle', was unknown – or only suspected. *Cinchona* as a tree was not known to Europeans, at least until its description by genuine botanists around 1730, so that fakes could easily be sold instead of the genuine product. Also, the search for less expensive alternatives to *Cinchona* contributed to market confusion. From the very beginning, both Jesuits and the Spanish crown attempted to control the quality of the material sent to, and sold in, Europe. It is worth noting that the genuine 'good' starting material (red and bitter bark) and a reproducible method for extracting its active principle was not established and publicised until the late 1730s. This goes some way to explaining large disparities in the reported efficacy of bark in malaria. Knowledge about that was empirically acquired largely by merchants and apothecaries. The scientific reasons for differences among barks and different parts of the tree were not identified until 1842, after a systematic survey of their quinine content by Bouchardat.[15]

By the middle of the 17th century bark as a treatment was becoming widely discussed, especially in the Protestant world, which was suspicious of any remedy promoted by the Jesuits: religious bigotry played a large part in the arguments that raged about the bark's efficacy.[12] This may have been the reason that Robert Talbor's 'discovery' of an effective treatment for ague (malaria), published in 1672 as *Pyretologia, or a rational account of the cause and cure of agues*, revealed no details of the treatment other than it had four ingredients, two of which were native to England.[13] Talbor may have been among the first practitioners to carry out clinical trials, and one of the reasons for his success may have been his access to the best species of bark through his smuggling contacts in Essex.[13]

Talbor's claims may have been one of the reasons that Thomas Sydenham (dubbed 'the English Hippocrates') may have changed his mind about the bark. In his first book on methods for curing fevers published in 1666, Sydenham was somewhat lukewarm about the role of bark. Ten years later, he endorsed it heartily while, by implication, condemning all other touted 'specifics': 'I am sure of this, that the only specific is the Peruvian bark', describing it as his 'sheet anchor'.[16] Sydenham's change of heart may have resulted from using an infusion of bark supplemented with tincture of bark (prepared according to Talbor's recipe) instead of crude bark powder.[14]

Formal evaluations of the effects of Cinchona *bark during the 18th century*

By the end of the 17th century, the notion that *Cinchona* bark was specific for fevers had become progressively firmly established, and several early therapeutic 'trials' have been documented, for example, at the Chinese court[17,18] and at the court of Louis XIV of France.[19,20]

In 1712, Franceso Torti, physician of the Duke of Modena, appears to have been the first to conduct systematic studies of the effects of *Cinchona* on different types of fever, and the first modern description of intermittent fevers.[21] He introduced the sensitivity and resistance of fevers to *Cinchona* bark in the nosography of fevers, anticipating the nosological usage of response to quinine used after 1820. For resistant fevers, he recommended giving up to a total of 20 drachms (2.5 ounces) spread over three weeks.[13] He summarised his representation of fevers by drawing a symbolic 'life tree' inspired by the *Cinchona* tree: on the left, the list of fevers resistant to bark extracts is

associated with flowers and leaves; on the right, intermittent fevers sensitive to quinine are listed and associated with bare branches. See: http://www.biusante.parisdescartes.fr/histoire/images/index.php?refphot=01803.

By the middle of the 18th century, it appears to have become widely accepted that *Cinchona* bark was useful in treating at least some fevers. For example, in a book on fevers published in 1763,[22,23] James Lind criticised the 'false manner of reasoning' that sometimes led a treatment to be rejected if it had not helped in a particular case:

> ... when, from a few exceptions, an attempt is made to overthrow the established maxims of the science. As, for example; if the bark should fail of curing an ague, or mercury of removing a venereal taint, are we thence rashly to conclude, that either of those medicines will prove, in all other instances and cases, ineffectual? (Lind,[22] p.56)

In the 1768 edition of 'An essay on diseases incidental to Europeans in hot climates', Lind leaves no doubt about his respect for bark, advising that:

> All naval and other sea surgeons, whose ships are bound to the East Indies, should take with them ten times the usual quantity of bark, and upon this account be excused from taking other drugs not wanted in that climate, as bark is procured there with great expense and difficulty. (Lind,[24] p.81)

One of the main difficulties in using *Cinchona* bark was the large variability of antimalarial effects of the preparations. This variation reflected the type of *Cinchona* tree, the place it was grown and several other variables that influenced the content of the active principle of the bark. McCausland,[25] a surgeon in the British Army stationed in Canada, reported having cured only 60 (64%) of 94 patients with Peruvian bark compared with 84 (82%) of 103 treated with tartar emetic (antimony potassium tartrate). Aware of the methodological shortcomings of his retrospective analysis, he mentioned that he thought that his comparison may not have been reliable: 'Solid and invariable conclusions', he suggested, 'can only be drawn from a very large number of experiments and observations[11] (see also McCausland,[25] pp.281–282).

Maehle[11] has commented on the way that McCausland and other late 18th century medical practitioners attempted to justify and refine therapeutic methods by providing comparative, quantitative information on their successes and failures with different forms of treatment. The quantitative, retrospective analysis of their therapeutic experiences, sometimes with a few, sometimes with hundreds of cases arranged in tables, helped practitioners to shape their preferred methods of treatment. For example, Home[26] compared giving bark before an anticipated fit with giving it after the fit had occurred, both in group comparisons and in within-patient 'crossover' trials (giving it after the fit seemed more effective); and Colingwood[27] compared different kinds of bark. A few years later, Robert Robertson[28] tabulated comparisons showing that only 1/216 (0.5%) Royal Navy sailors with 'ship fever' died after receiving bark compared with 19 among 296 (6.4%) receiving antimony and other treatments.[11] As the fevers were much more likely to be due to typhus than malaria, the difference is more likely to have reflected the toxicity of antimonials than the efficacy of bark.

Part 2: Synthesis and evaluation of quinine during the 19th and 20th centuries

In the light of the increasing number of positive medical reports which had accumulated by the beginning of the 19th century, few doubted that *Cinchona* bark was a 'good' medicine for intermittent fevers. After 1800, improvement in procedures for acid–base extraction of the active principles of medicinal plants yielded a number of purified molecules of medical interest. The treatment of several diseases, formerly based on the use of crude plant extracts, could now be examined on a more quantitative basis. As a consequence, there was an explosion of clinical experiments with novel molecules.[29] After quinine and other *Cinchona* alkaloids had been purified in 1820,[30] the molecule was promptly tested in patients. Numerous medical observations and case reports from all over the world soon indicated that quinine was specific for 'malarial' (intermittent) fevers. Treatment of fevers with quinine thus contributed markedly to the nosography of malaria by distinguishing between fevers responding to the extracts and those that did not.

Magendie[29] first used dogs to check whether there was any significant toxicity associated with quinine and its salts. John Elliotson,[31] a physician at St. Thomas' Hospital in London, provided an account in English of Magendie's and other French experience:

> As soon as the two alkalies of Cinchona were discovered, M. Pelletier sent a quantity to Dr. Magendie, who administered them to dogs in large doses without nausea, vomiting, or other apparent result. The indefatigable and acute physiologist

> then injected into the veins of these animals from two to ten grains of the sulphate and of the acetate of Quinine and Cinchonina in solution, but with no more effect. Satisfied of the innocence of the substances, he ordered the sulphate of quinine to several scrofulous children affected with ulcers [possibly tuberculosis of the lymph glands], and in a fortnight the most decided benefit was obtained.

After Magendie had satisfied himself that quinine was not toxic in humans, it was widely tested in France in hospital patients suffering from intermittent fevers.[31] Quinine – mostly used as a sulphate, tannate or acetate salt – met with the same success as bark extracts. However, questions were asked about whether it was worth using a costly purified material instead of widely used crude bark extracts.

A fairly definitive answer in favour of quinine came from John Elliotson's review of the experience of French physicians – particularly Double[32] and Chomel and Villermé[33] (reviewed in Rouzet)[34] – and his own assessment of the effects of free base or sulphate salt of quinine in 16 adult patients in Britain who had intermittent (tertian and quartan) fevers. Elliotson[31] provided a medical and social history of each of them, including information about unsuccessful previous treatments. He gave each of them 5 grains of quinine or quinine sulphate every 6 h; noted the rapid decrease in the intensity of rigors and continued until there had been no rigor for a fortnight. No difference was perceived between those who had received quinine and those who had received its salt. Some side effects were noted, including vomiting, but none was such that the treatment had to be discontinued.

Elliotson's conclusion was straightforward: quinine or its salts should be used instead of bark extracts to treat intermittent fevers. He stated the advantages of quinine over bark as follows:

> Quinine is nothing but the new form of an old medicine, but presented in such a way that no intermittent fever can resist it . . . It is very true that Quinine and Cinchonina cannot strictly be called new medicines, because they exist, one or both, in the Cinchona which we have all been prescribing. We are in the situation of M. Jourdain, in Molière's Bourgeois Gentilhomme, who had been speaking prose all his life without knowing it . . . But although we have not gained a new medicine, the acquisition of so compendious a form of bark, if one may so speak, is highly important . . . The patient has only to take a pill, and is spared the annoyance of swallowing any of the mass of inert powder which remains after the extraction of Quinine, and which frequently, whatever may be the disease, so disgusts him, or so oppresses his stomach, and deranges his system at large, that bark cannot be borne in efficient quantity, or at all: and, what is particularly interesting, we find that they succeed when bark has failed – that they cure cases of intermittent fever which have resisted bark, although perfectly well borne, and freely administered.

Elliotson tended to prefer the free base of quinine, because it involved fewer preparation steps and so was less expensive. Some of his comments on cured cases also implied that treated patients were protected against further fever attacks.

A few years later, Magendie[29] summarised and discussed a larger number of case reports, alongside similar pharmacological studies of other alkaloids. He mentions the difficulties in defining intermittent fever as a nosographical entity common to all studies, but, on the basis of many publications from various countries (Great Britain, Ireland, Italy and France, for example), he concludes that quinine was indeed highly effective against intermittent fevers. Some of the Italian case series he quoted included up to 64 patients with different forms of severe intermittent fevers. The outcome of the treatment was compared to previous experience of treatment with bark extracts, or to what was known to occur in the absence of treatment. Recovery was defined as the disappearance of clinical signs and the absence of relapse in the short term. In that respect, the list of cases of intermittent fevers treated with quinine, as collected by Magendie,[29] appears as a kind of success story: so far, no failure had been noted.

The notion of intermittent fevers 'being cured by quinine' has to be tempered by claims made for the use of quinine in other disorders. Magendie notes that several authors reported using quinine to treat or alleviate a variety of non-malarial diseases – ulcers, haemorrhoids, gastric inflammation, intermittent neuralgia, and haemoptysis, for example. All of these diseases are characterised by some periodicity, as if 'intermittence' itself was more of a cause than a consequence of the disease.[35] In any case, Magendie's thinking probably reflected the still prevalent view that 'intermittent disorders' in general were caused by inflammation of internal organs, particularly the stomach and the bowel, which itself was due to some unspecified intermittent miasmatic influence.

In the years that followed, quinine was very widely used as a specific treatment for intermittent fevers, albeit, not universally. For example, based on his investigations in Algeria, Boudin[36] judged arsenic oxide to be a preferable antimalarial to quinine. Numerous preparations contained either quinine alone or combined with alkaloids like morphine, or

with arsenic or tartar emetic (antimony potassium tartrate). Jourdan's *Pharmacopée universelle*[37] describes more than 100 official preparations based on quinine, bark powder or bark extracts, all believed to be endowed with distinct properties on a huge variety of diseases, for example, typhus,[38] or used as a tonic.[39] Like arsenic,[40] quinine became a kind of panacea.

Choice of quinine dose for treating malaria

How was an appropriate dose of quinine first defined? The dose of quinine used in 1820 and in later studies appears to have been adapted from the quantity of bark powder previously used for intermittent fevers. Depending on the origin of the bark, the content as quinine lies in the range of 1–4% of dry material.[29,39,41] For example, at the court of Louis XIV,[19,20,42] 'pre-quinine' physicians had routinely used 1.5–2 ounces of bark every day for a minimum of six days, as powder, extract or pills. This is in the range of 1–2 g of quinine a day. A rough computation based on more accurate prescriptions for the preparation of bark powder potions for fevers[37] suggests daily doses equivalent to about 1 g of quinine, distributed in several doses. Such a computation is obviously subject to substantial uncertainty, particularly concerning the concentration of quinine in the original material. It suggests, however, that the daily doses used after 1820 were merely adaptations from a century and a half of use of bark powder.

Not only did isolation of quinine offer patients a more convenient way of being treated, it offered physicians the possibility of treating malaria using a quantified protocol for drug administration. The dose of pills of quinine to be administered was established after the first wave of trials on humans between 1820 and 1822. Most authors mentioned by Magendie[29] used an initial 3–5 grains of quinine powder in pills. The administration was repeated, usually no more frequently than twice a day, until complete disappearance of rigors. Elliotson kept to 5 grains every 6 h. Italian doctors reported the daily use of up to 25 grains to treat severe and relapsing intermittent fevers. Despite the variations and uncertainties in the reports, Magendie[29] gathered evidence that looks like a crude dose–response study: less than 2 grains a day was ineffective and more than 15 grains a day was found to be either actually toxic (leading to tinnitus and vomiting, for example) or simply badly tolerated by patients. Assuming that quinine preparations were pure and identically prepared – which is far from certain – consensus on the treatment of intermittent fevers rested on the administration of 5 to 15 grains of quinine per day, usually in several doses, although with noticeable local variations.

The range of prescribed doses endured. Eighty years later, medical text books, including Manson's *Tropical Medicine*,[43] recommended doses across a similar range. Depending on the authors and the forms of malaria, 1 g of quinine sulphate, chlorhydrate or tannate was administered before the acute phase, followed by smaller daily doses, and 0.25 to 0.4 g per day in the longer term. Later on, there was some variation in treatment protocols depending on the *Plasmodium* species targeted and discussion about dosage continued;[44] but there was actually little change in protocols over time. The effects of different doses of quinine in neuro-syphilis patients infected with malaria were studied alongside mean plasma quinine concentration. The 'class of therapeutic effect' – a measure of efficacy in terms of parasite presence and concentration in the blood – provided estimates of dosage, mean quinine concentration and therapeutic effect in a case series of patients.[45] By contrast with dosage, the duration of treatment continued to vary greatly, ranging from a few days to months.

Side effects of bark, particularly nausea and vomiting, were recognised in the 17th century. Cinchonism – a combination of tinnitus, high-tone hearing impairment and nausea – was clearly defined by Magendie[29] after he had administered pure quinine to dogs and later to patients. Case studies published after 1830 report hearing loss, headaches, vertigo, disturbances of vision, bradycardia and digestive problems. Different alkaloids were compared to identify which had fewest of these problems while retaining antimalarial effectiveness.[13] Very high doses of bark and later quinine were used in fevers that were unresponsive to lower doses. One medical dictionary considered (unspecified) large doses of quinine as about as poisonous as morphine,[46] and Dechambre's dictionary[47] contains a lengthy discussion of the side effects of large doses (up to 4 g) of quinine. Honigsbaum and Wilcox[13] conclude that a combination of the alkaloids at the same total dose (but a lesser dose of each individual component) reduces the frequency of at least some of the adverse effects.

Controlled trials to compare antimalarial drugs in the early 20th century

In 1866, the Madras Government appointed a Commission to examine the respective efficacy of different alkaloids in treating malaria. In an article published some time later, Dymock, Warden and Hooper[48] wrote:

> From the Report it appears that the number of cases of paroxysmal malarious fevers treated was 2,472,

> – namely, 846 with quinine, 66 with quinidine, 559 with cinchonine and 403 with cinchonidine. Of these 2472 cases, 2,445 were cured and 27 failed. The difference in remedial value of the four alkaloids as deduced from these experiments may be thus stated:
>
> Quinidine ratio of failure per 1000 6
> Quinine " " " 7
> Cinchonidine " " " 10
> Cinchonine " " " 23

Dymock and his colleagues do not report whether the Commission's 'experiments' were 'natural' or 'planned'.

Quinine alkaloids were not the only drugs tested as antimalarials, however. After Paul Ehrlich had discovered that some dyes stained microorganisms, methylene blue became used as an antimalarial.[49] A brief contemporary report of a controlled comparison of methylene blue with quinine concluded that the former was superior,[50] and interest in methylene blue continues today.[51] The introduction of methylene blue led to chemical substitutions of the original aromatic nucleus and identification of a number of other molecules active against malaria (most of which were not tested until the 1930s – see below). In addition, as arsenic and arsenic derivatives had long been used as antimalarials, organic derivatives of arsenic (Atoxyl and Salvarsan) developed to treat syphilis[52] were also tested in malaria patients.[44] However, the studies were poorly controlled and small scale, and none of the molecules proved sufficiently active to dislodge quinine and its alkaloids from their dominant position in the treatment of malaria.

Planned, alternate allocation to treatment comparison groups began to be used in India at the end of the 19th century in controlled evaluations of interventions to prevent and treat cholera and plague.[53–56] Over the first two decades of the 20th century, the criteria for treatment tests in other diseases, including malaria, began to be developed. Acton[57] reported his research on the treatment of malaria in India using different quinine alkaloids. He mentions that he had consulted the statistician Karl Pearson and the physiologist Henry Dale and made the following recommendations:

1. The population under investigation should be sufficiently large and homogeneous. At least 100 men should be treated, and the parasites must be of the same species and found microscopically in every case.
2. Reinfections must be excluded during treatment and observation.
3. Eight weeks should be the minimum period of observation required after treatment.
4. The experiment should be repeated if necessary to eliminate the errors due to chance distribution.

An important omission from Acton's list of conditions was the need to generate groups of patients who were otherwise comparable by using alternate (or random) allocation to the treatments being compared. Uncertainty about the validity of Acton's conclusions appears to have been one of the reasons that the British Medical Research Council decided that it was important to check Acton's conclusion that quinidine and cinchonidine were outstandingly effective in achieving a permanent cure of benign tertian malaria.[57] A *Cinchona* Derivatives and Malaria Committee was formed and one of its eight requirements for comparisons was that: '*Alternate patients will be treated with quinine and quinidine respectively, until 50 cases have been treated with each*'.[58]

The Committee made arrangements for 'confirmative trials' in Khartoum, El-Obeid, Lagos, Port of Spain, Georgetown, Dar es Salam, Nairobi, Entebbe and Kuala Lumpur, using the disappearance of parasites from the peripheral blood as the principal measure of effect.

The hoped-for data were not forthcoming from most of the centres. By far, the most complete information (from 72 patients alternately allocated to quinine or quinidine) was supplied by William Fletcher[59] from the Institute for Medical Research at Kuala Lumpur, in the Malay States. Seventeen years earlier, Fletcher had used alternate allocation to create comparable groups of asylum inmates in a comparison of the effects of unpolished and polished rice on the incidence of beri-beri.[60,61] In his report of a controlled comparison of quinine and quinidine for the British Medical Research Council, Fletcher described his use of a similar approach[59]:

> The patients were not selected in any way; all who came into the hospital with malaria were treated with purified drugs until the supply was exhausted. Seventy-two patients were numbered consecutively on their admission to hospital. The first patient and all those bearing odd numbers were given quinine; those with even numbers (i.e. the second and alternate patients) were given quinidine.

Fletcher concluded that the immediate effect of quinidine bisulphate 'is as good as, or slightly better that quinine bisulphate'. However, as illustrated by a controlled comparison of the two drugs reported in 1932,[62] uncertainties about their relative merits persisted; and even today, quinidine remains a parenteral drug used for severe malaria in the USA, where quinine is often not available.[63]

Although Fletcher had used alternation twice in treatment evaluations early in the history of controlled trials, he does not appear to have adopted this as a routine design feature in his research on treatments for malaria at the Institute for Medical Research.[64] However, others at the Institute used alternate allocation to compare quinine with different quinine alkaloids and mixtures of alkaloids[65,66] and with the new synthetic antimalarial drugs such as atebrin.[67,68]

In 1926, the *Indian Journal of Medical Research* published the first of a series of 11 reports of research on the treatment of malaria by Major John Alexander Sinton. In the first article,[69] Sinton itemises the steps necessary to test the efficacy of any treatment:

> It is very necessary in conducting experimental investigations into the effects of any drug in the treatment of malarial fevers that strict precautions should be taken to ensure:
>
> 1. that the disease being treated is malaria, diagnosed not merely by clinical signs and symptoms, but by the finding of the parasites immediately before the commencement of treatment;
> 2. that the patient has no other disease, the signs and symptoms of which might obscure the effects of the drug being tested;
> 3. that the drug being tested is actually being taken and retained in the amounts prescribed;
> 4. that no other drug is being taken at the same time, which might vitiate the results of the experiments;
> 5. that, in comparing different treatments, infections due to the different species of malaria parasite are considered separately;
> 6. that in comparing the effects of one treatment with another, the populations treated by the different methods should be as far as possible homogenous;
> 7. that a sufficient number of patients are treated, in order that the results may not be vitiated by errors of chance distribution;
> 8. that controls are used to eliminate, as far as possible, any possible variations in the results, due to season, virulence of the parasites, immunity, etc.;
> 9. that a strict standard as to what is to be considered as a 'cure' of the infection is laid down; and
> 10. that if this standard depends on a period of observation, chances of re-infection are excluded during this time.

In subsequent reports, Sinton reports that 'The alternative case control method was used, and the cases as diagnosed were placed alternately in a control and an experimental group so that there should be no bias in the choice of patients on account of any apparent severity or otherwise in the infection';[70] 'patients were allotted to the different series in strict rotation to avoid any personal bias in the selection of the cases'.[71] In the last report in the series,[72] Sinton reiterates an essential feature of his studies:

> In all tests at least two treatments, of which one was a quinine treatment, were carried on at the same time, so that the result of one treatment might act as a control on the other. In several instances, three or even four forms of treatment were conducted during the same period, the alternative case method being used.

As in other spheres at the time,[55] alternation was increasingly adopted during the 1930s as a feature of controlled comparisons of alternative antimalarial treatments. A large multicenter comparison of quinine and Totaquina (a mixture of *Cinchona* bark alkaloids) was conducted under the auspices of the League of Nations involving participants in Algeria, Bulgaria, China, France, Italy, the Federated Malay States, Morocco, Roumania and Spain.[73] Although the report refers to the need to 'standardise so far as possible the technique of the experiments and to prevent any selection of cases likely to give misleading results', there is no mention of the method used to allocate patients to the comparison groups, and no suggestion from the Tables that alternation was used. By contrast, Hicks and Diwan Chand[74] reported that in their study 'quinine sulphate and Totaquina, types I and II, were given to alternate cases of benign tertian, and similarly to alternate cases of malignant tertian'.

Summarising their assessment of these and similar studies done during the 1920s and early 1930s, Honigsbaum and Wilcox[13] concluded that the crude extracts of *Cinchona* bark were as effective as quinine in the treatment of both vivax and falciparum malaria and might be even more effective in dealing with quinine resistant *Plasmodium falciparum*. A limitation of these and later controlled studies was that the treatment benefit was assessed only by parasite clearance in the short term.

Alternation was also used in some of the comparisons of quinine with new synthetic antimalarial drugs in studies organised under the aegis of the Malaria Commission of the League of Nations.[75] As noted earlier, controlled comparisons of quinine with atebrine were done in the Malay States.[67,68] In Bolshevo in the Soviet Union, all patients suffering from acute attacks were given either quinine, acriquine, quinine plus plasmocide or acriquine plus plasmocide 'in the

order of their arrival at the dispensary' (p.1066). Alternation was also used in comparisons of atebrine alone with atebrine and plasmoquine/chloroquine[76] and alternation may have been used by Mezincesco and Cornelson in Roumania (p.979). Swellengrebel's and de Buck's studies in the early 1930s assessing the prophylactic efficacy of plasmoquine in previously healthy volunteers in the Netherlands,[77,78] and Soesilo's and Gilbert's[79] similar assessment of atebrin given prophylactically among volunteers in the Dutch East Indies, were small scale and contributed little to an understanding of the potential use of these agents in field conditions. Alternate allocation trials involving quinine continued to be reported several years after the end of World War II (see, for example, Johnstone)[80] until concealed random allocation became widely adopted as the most secure way of preventing allocation bias.[55]

Declarations

Competing interests: None declared.

Funding: None declared.

Ethics approval: Not applicable.

Guarantor: GG

Contributorship: GG, EF, UT and IC, were responsible for researching and reviewing sources in French, Italian, German and English, respectively. PG ensured that the article was malariologically accurate. All authors agreed the final version of the article.

Acknowledgements: The authors thank Nor Asiah, Estela Dukan, Christoph Gradmann, Ronald Henry, Su May Liew and Holger Maehle for providing information; Jan Peter Verhave for information about Nicolaas Swellengrebel; Mohamed Asyraf Bin Noh for help in locating relevant material in Kuala Lumpur; and Nicholas White for helpful comments on an earlier draft.

Provenance: Invited article from the James Lind Library.

References

1. Celsus AC (beginning 1st century). *De Medicina*. French translation by Dr. Védrènes (Traité de medicine). Paris: Masson, 1876.
2. Peter JP. Malades et maladies à la fin du XVIIIe siècle [Patients and diseases at the end of the XVIIIth century]. In: Desaive JP, et al. (eds) *Médecins, Climat et Épidémies à la Fin du XVIIIè Siècle*. Paris: Mouton La Haye, 1972.
3. Alibert JL. *Traité des Fièvres Pernicieuses Intermittentes*. [*Treatise on intermittent pernicious fevers*], 2nd ed. Paris: Crapart, Caille et Ravier, 1804.
4. Coster J. *Dictionnaire de Santé ou Vocabulaire de Médecine*. [*Dictionary of health or vocabulary of medicine*]. Paris: Gabon, 1829.
5. Malaria Commission. Report on its tour of investigation in certain European countries in 1924 (League of Nations). Report CH273. Geneva: Health Organization, 1925.
6. Verhave JP. *The Moses of Malaria. Nicolaas H. Swellengrebel (1885-1970) Abroad and at Home*. Rotterdam: Erasmus Publishing, 2011.
7. Ephraim A. Uber die Bedeutung de statistischen Methode für die Medicin [On the relevance of the statistical method for medicine]. Volkmann's Sammlung Klinische Vortraege N.F. *Innere Medicin* 1890; 24: 706–716.
8. Gachelin G. The Interaction of Scientific Evidence and Politics in Debates about Preventing Malaria in 1925. JLL Bulletin: Commentaries on the history of treatment evaluation. See www.jameslindlibrary.org/articles/the-interaction-of-scientific-evidence-and-politics-in-debates-about-preventing-malaria-in-1925/ (2013, last checked 12 November 2016).
9. Baldi S. *Anastasis Cortices Peruviae, Seu Chinae Chinae Devensio, Gênes, Typis Petri Ioannis Calenzani*. See http://gallica.bnf.fr/ark:/12148/bpt6k511115.r=anastasis%20corticis= (1663, last checked 12 November 2016).
10. Jarcho S. *Quinine's Predecessor: Francesco Torti and the Early History of Cinchona*. Baltimore: Johns Hopkins University Press, 1993.
11. Maehle A-H. Four early clinical studies to assess the effects of Peruvian bark. JLL Bulletin: Commentaries on the history of treatment evaluation. See www.jameslindlibrary.org/articles/four-early-clinical-studies-to-assess-the-effects-of-peruvian-bark/ (2011, last checked 12 November 2016).
12. Rocco F. *The Miraculous Fever Tree: The Cure that Changed the World*. London: Harper Collins, 2004.
13. Honigsbaum M and Willcox M. Cinchona. In: Willcox M, Bodeker G, Rosoanaivo P (eds) *Traditional medicinal plants and malaria*. Boca Raton: CRC Press, 2004, pp.21–41.
14. Boumediene S. Avoir et savoir. L'appropriation des plantes médicinales de l'Amérique espagnole par les Européens (1570–1750). [Possession and knowledge. The appropriation by Europeans of medicinal herbs from Spanish America]. PhD Thesis, Université de Lorraine, 2013. See www.theses.fr/2013LORR0345 (last checked 13 November 2016).
15. Delondre A and Bouchardat A. *Quinologie. Des quinquinas et des questions qui dans l'état present de la science et du commerce s'y rattachent avec le plus d'actualité* [Cinchonology: Cinchonas and the questions that are most relevant in the current state of science and trade]. Paris: G Baillière, 1854.
16. Cook H. Markets and cultures: medical specifics and the reconfiguration of the body in early modern Europe. *Trans R His Soc* 2011; 21: 123–145.
17. de Fontaney F. Letter to F de la Chaise, from Cheu-Chan (a port in the Province of 'Chekian'), dated 15 February. In: *Lettres édifiantes et curieuses, écrites des Missions Étrangères par quelques missionnaires de la Compagnie de Jesus* [Edifying and curious letters, written from the foreign missions by some missionaries of the Company of Jesus]. 34 vols. Paris: Chez Nicolas le Clerc, rue Saint Jacques, à l'Image Saint Lambert, 1703–1776. Vol. 7 (1707):234–244.
18. Cook H. Testing the effects of Jesuit's bark in the Chinese Emperor's court. JLL Bulletin:

Commentaries on the history of treatment evaluation, 2010. See www.jameslindlibrary.org/articles/testing-the-effects-of-jesuits-bark-in-the-chinese-emperors-court/ (last checked 13 November 2016).
19. Vallot A, D'Aquin A and Fagon GC. *Journal de la santé du roi Louis XIV de 1647 à 1711* [*Diary of the health of King Louis XIV from 1647 to 1711*]. Paris: Auguste Durand (manuscript completed by Fagon dated 1711, published in 1862). See http://gallica.bnf.fr/ark:/12148/bpt6k203302w.r=Journal%20de%20la%20sant%C3%A9%20du%20roi%20Louis%20XIV%20de%20l'ann%C3%A9e (last checked 13 November 2016).
20. Ferez S. Louis XIV et le quinquina [Louis XIV and cinchona]. *Vesalius IX* 2003; 2: 25–30. See www.biusante.parisdescartes.fr/ishm/vesalius/VESx2003x09x02x025x030.pdf (last checked 13 November 2016).
21. Torti F. *Therapeutice Specialis ad Febres quasdam Periodicas Perniciosas*. Modena: B Soliani, 1712.
22. Lind J. *Two Papers on Fevers and Infection*. London: D Wilson, 1763.
23. Tröhler U. James Lind at Haslar Hospital 1758–1774: a methodological theorist. JLL Bulletin: Commentaries on the history of treatment evaluation, 2003. See www.jameslindlibrary.org/articles/james-lind-at-haslar-hospital-1758-1774-a-methodological-theorist/ (last checked 13 November 2016).
24. Lind J. *An Essay on Diseases Incidental to Europeans in Hot Climates*. London: Becket and De Hondt, 1768.
25. McCausland R. Facts and observations on different medical subjects. *Med Comment* 1783; 8: 247–296.
26. Home F. *Clinical Experiments, Histories and Dissections*. London: Murray, 1780.
27. Colingwood T. Observations on the Peruvian Bark. *Med Comment* 1785; 10: 265–275.
28. Robertson R. *Observations on Jail, Hospital, or Ship Fever*. New ed. London: J. Murray, 1789.
29. Magendie F (1822 [1829, 2nd edn]). *Formulaire pour la préparation et l'emploi de plusieurs médicaments* [*Formulations for the preparation and the usage of several drugs*]. Paris: Imprimerie de Plassan. See http://gallica.bnf.fr/ark:/12148/bpt6k5832713d.r=Magendie%2C+Fran%C3%A7ois.langEN (last checked 13 November 2016).
30. Pelletier J and Caventou JB. *Analyse Chimique des Quinquinas* [*Chemical analysis of cinchonas*]. Paris: C Colas, 1821.
31. Elliotson J. Illustrations of the medical properties of quinine. *Med-chirurg Trans* 1823; 12: 544–564.
32. Double FJ. *Considérations thérapeutiques sur une nouvelle préparation de quinquina* (Therapeutic considerations on a new preparation of cinchona). Revue médicale, 6ème livraison, 1820.
33. Chomel AF and Villermé LR. Sur l'emploi de la quinine et de la cinchonine sur les fiévres intermittentes. [On the use of quinine and cinchonine for intermittent fevers] *Journal de Pharmacie* 1821; 135–139.
34. Rouzet M. Observations médicales sur l'emploi des bases salifiables des quinquinas [Medical observations concerning the use of neutralized bases extracted from cinchonas]. *J Pharm* 1821; 128–133.
35. Sigaud J-Fr-X. *Du climat et des maladies du Brésil ou Statistique médicale de cet Empire* [*About the climate and diseases of Brazil, or medical statistics of this Empire*]. Paris: Fortin et Masson, 1844.
36. Boudin JChM. *Traité des fieèvres intermittentes, reémittentes et continues des pays chauds et des contreées mareécageuses suivi de recherches sur l'emploi theérapeutique des preéparations arseénicales* [*Treatise on the intermittent, remittent and continuous fevers of hot climates and marshes, followed by research on the therapeutic use of arsenic-containing preparations*], Germer-Baillieère, Paris, 1842.
37. Jourdan AL. *Pharmacopée universelle, ou conspectus des pharmacopées de…* [*Universal pharmacopeia, or conspectus of pharmacopeias from…*]. Paris and London: Baillière JP, 1840.
38. Thompson TRH. On the value of quinine in African remittent fever. *Lancet* 1846; 1: 244–245.
39. Dorvault. *L'officine ou répertoire général de pharmacie pratique*. (Pharmacy, or general formulary for practical pharmacy), 5ème édn. Paris: Labé, libraire de la faculté de medicine, 1858.
40. Whorton JC. *The Arsenic Century. How Victorian Britain was Poisoned at Home, Work and Play*. Oxford: Oxford University Press, 2010.
41. Delondre A and Bouchardat A. *Quinologie. Des quinquinas et des questions qui dans l'état present de la science et du commerce s'y rattachent avec le plus d'actualité* [*Cinchonology: Cinchonas and the questions that are most relevant in the current state of science and trade*]. Paris: G Baillière, 1854.
42. de Blégny N. *La découverte de l'admirable remède anglois pour la guérison des fièvres* [*The discovery of the admirable English remedy for the cure of fevers*]. Paris, Blageart/D'Houry, 1680.
43. Manson P. *Tropical Diseases*. London: Cassell, 1898.
44. Marchoux E. *Les traitements du paludisme* [*Treatments of malaria*]. Malaria Commission CH/Malaria/193, 1933.
45. Earle DP Jr, Berliner RW, Taggart JV, Welch WJ, Zubrod CG, Wise NB, et al. Studies on the chemotherapy of the human malarias. II. Method for the quantitative assay of suppressive antimalarial action in falciparum malaria. *J Clin Investig* 1948; 27: 75–79.
46. Adelon NP. *Dictionnaire de médecine ou répertoire général des sciences médicales considérées sous le rapport théorique et pratique* [*Dictionary of medicine, or general index of medical sciences considered in the light of the relation between theory and practice*]. Paris: Béchet jeune, 1842.
47. Dechambre A. *Dictionnaire encyclopédique des sciences médicales*. Paris: G. Masson et P. Asselin, 1877.
48. Dymock W, Warden CJH and Hooper D. A history of the principal drugs of vegetable origin met with in British India. *Pharmacographia Indica Vol. II*. London: Kegan Paul, Trench, Trübner, 1891, pp.180–181.
49. Guttmann P and Ehrlich P. Über die Wirkung des Methylenblau bei Malaria (On the effect of methylene

blue on malaria). *Berliner Klinische Wochenschrift* 1891; 28: 953–956.
50. Maitland CB. Methylene blue in malarial fever. *BMJ* 1900; 2: 532–533.
51. Meissner PE, Mandi G, Coulibaly B, Witte S, Tapsoba T, Mansmann U, et al. Methylene blue for malaria in Africa: results from a dose-finding study in combination with chloroquine. *Malaria J* 2006; 5: 84.
52. Williams KJ. The introduction of 'chemotherapy' using arsphenamine – the first magic bullet. JLL Bulletin: Commentaries on the history of treatment evaluation, 2009. See www.jameslindlibrary.org/articles/the-introduction-of-chemotherapy-using-arsphenamine-the-first-magic-bullet/ (last checked 13 November 2016).
53. Choksy NH. Professor Lustig's plague serum. *Lancet* 1900; 2: 291–292.
54. Haffkine WM. On preventive inoculation. *Proc R Soc Lond* 1900; 65: 252–271.
55. Chalmers I, Dukan E, Podolsky SH and Davey Smith G. The advent of fair treatment allocation schedules in clinical trials during the 19th and early 20th centuries. JLL Bulletin: Commentaries on the history of treatment evaluation. See www.jameslindlibrary.org/articles/the-advent-of-fair-treatment-allocation-schedules-in-clinical-trials-during-the-19th-and-early-20th-centuries/ (last checked 13 November 2016).
56. Ramanna M. Nasarwanji Hormusji Choksy (1861-1939), a pioneer of controlled clinical trials. JLL Bulletin: Commentaries on the history of treatment evaluation, 2014. See www.jameslindlibrary.org/articles/nasarwanji-hormusji-choksy-1861-1939-a-pioneer-of-controlled-clinical-trials/ (last checked 13 November 2016).
57. Acton HW. Researches on the treatment of benign tertian fever. *Lancet* 1920; 1: 1257–1261.
58. Medical Research Council. Report of the committee upon cinchona derivatives and malaria. In: *Clinical Comparisons of Quinine and Quinidine*. London: HMSO, 1925, pp.5–16.
59. Fletcher W. The relative efficiency of quinine and quinidine in the treatment of malaria. In: *Medical Research Council. Clinical Comparisons of Quinine and Quinidine*. London: Her Majesty's Stationary Office, 1925, pp.17–27.
60. Fletcher W. Rice and beri-beri: preliminary report on an experiment conducted in the Kuala Lumpur Insane Asylum. *Lancet* 1907; 1: 1776–1779.
61. Vandenbroucke JP. The contribution of William Fletcher's 1907 report to finding a cause and cure for beriberi. JLL Bulletin: Commentaries on the history of treatment evaluation, 2003. See www.jameslindlibrary.org/articles/the-contribution-of-william-fletchers-1907-report-to-finding-a-cause-and-cure-for-beriberi/ (last checked 13 November 2016).
62. Sanders JP and Dawson WT. Efficacy of quinidine in malaria. *JAMA* 1932; 99: 1773–1777.
63. White NJ, Looareesuwan S, Warrell DA, Chongsuphajaisiddhi T, Bunnag D and Narinasuta T. Quinidine in falciparum malaria. *Lancet* 1981; 2: 1069–1071.
64. Fletcher W. *Notes on the Treatment of Malaria with the Alkaloids of Cinchona*. Studies from the Institute for Medical Research, Kuala Lumpur, Federated Malay States No. 18. London: John Bale, Sons, and Danielsson, Ltd, 1928.
65. Institute for Medical Research. *Annual Report for the Year 1932*. Kuala Lumpur, 1933.
66. Institute for Medical Research. *Annual Report for the Year 1933*. Kuala Lumpur, 1934.
67. Field JW. The substitution of atebrin for quinine in treated "ambulatory" cases of malaria on rubber estates. In: Neave Kingsbury A (ed.) *Annual Report of the Institute for Medical Research, Federated Malay States*. 1934, pp.16–24.
68. Field JW, Niven JC and Hodgkin EP. The prevention of malaria in the field by the use of quinine and atebrine. *Bull Health Org League Nations* 1937; 6: 236–290.
69. Sinton JA. Studies in malaria, with special reference to treatment. Part I. Introduction and routine methods. *Ind J Med Res* 1926; 13: 565–577.
70. Sinton JA. Studies in malaria, with special reference to treatment. Part II. The effects of treatment on the prevention of relapse in infections with *Plasmodium falciparum*. *Ind J Med Res* 1926; 13: 579–601.
71. Sinton JA. Studies in malaria, with special reference to treatment. Part VII. The intravenous injection of sodium stovarsol in the treatment of benign tertian malaria. *Ind J Med Res* 1927; 15: 287–299.
72. Sinton JA and Bird W. Studies in malaria, with special reference to treatment. Part XI: the cinchona alkaloids in the treatment of benign tertian malaria. *Ind J Med Res* 1929; 16: 725–746.
73. Pampana EJ. Clinical tests carried out under the auspices of the Malaria Commission. *Bull Health Org League Nations* 1933; 3: 328–343.
74. Hicks EP and Diwan Chand S. The relative clinical efficacy of totaquina and quinine. *Records Malaria Surv Ind* 1935; 5: 39–50.
75. Malaria Commission. The treatment of malaria: study of synthetic drugs, as compared with quinine, in the therapeutics and prophylaxis of malaria. *Bull Health Org League Nations* 1937; 6: 897–1033.
76. Bastianelli G, Mosna E and Canalis A. Prevention and treatment of malaria by synthetic drugs. *Bull Health Org League Nations* 1937; 6: 822–891.
77. Swellengrebel NH and de Buck A. Prophylactic use of plasmoquine in a dosage warranting reasonable safety for routine treatment. *Proc Koninklijke Akademie van Wetenschappen* 1931; 35: 1216–1220.
78. Swellengrebel NH and de Buck A. Plasmoquine prophylaxis in benign tertian malaria. *Proc Koninklijke Akademie van Wetenschappen* 1932; 35: 911–914.
79. Soesilo R and Gilbert A. Report about malaria prophylaxis and treatment with atebrine. *Mededelingen Dienst der Volksgezondheid in Nederlandsch-Indie* 1934; 23: 5–7.
80. Johnstone RDC. Paludrine in relapsing benign tertian malaria: further trials. *Lancet* 1947; 1: 674–675.