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REVIEW ARTICLE

A Recent Approach for Developing an Anti-malarial Phytomedicine

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ABSTRACT

A reverse pharmacology approach to developing an anti-malarial phytomedicine is designed and implemented in Mali resulting in a new standardized herbal anti-malarial after six years of research. The first step is to select the remedy for development through a retrospective treatment. The second step is the dose-escalating clinical trial that showed a dose-response phenomenon and help the safe and most effective dose. The third step is to compare the phytomedicine in the first line treatment. The fourth step is to identify the active compound can used for standardization and quality control.

Keywords: Anti-malarial, Phytomedicine, Recent.

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INTRODUCTION

Malaria elimination efforts lead to much wide ruse of the few currently effective anti-malarial drugs, such as artesunate amodiaquine, artesunate sulphadoxine-pyrimethamine (SP), and artemether/lumefantrine. There is already discussion on intermittent presumptive treatment of infants, children, pregnant women, and even mass drug administration in some settings. Resistance already exists to amodiaquine and SP, are increase because of the increased drug pressure. The first signs of resistance to artemisinin derivatives are appearing in Cambodia.

In this context it is important to maximize the life- span of existing anti-malarials, and to consider all options for the development of new anti-malarials. Traditional medicinal plants have provided the source of the two major families of anti-malarial drugs artemisinin and quinine, so many researchers are screening plants for novel chemical entities to develop as "lead compounds" for new anti-malarial drug. However conventional drug development is poorest patients in remote areas, unless they are part of a heavily subsidized scheme.

In contrast the parallel development of standardized phytomedicines can be done faster, more cheaply, and more sustainably for remote areas. They could then be proposed and evaluated as a complement to existing strategies, for example as first aid in remote areas in case there is some delay until ACT treatment can be started. Their use might also delay the development of resistance to current standard drugs. The concept of "reverse pharmacology" is coined in India to develop pharmaceuticals from Ayurvedic medicines, and was also championed by the Chinese in the 1950s ,

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To develop a standardized phytomedicine, a "reverse pharmacology" approach is tested, where clinical evaluation was prioritized from the start. Isolation of compounds is done only at the end of the pathway, Anti-malarial phytomedicine from a traditional herbal remedy, namely *Argemone mexicana* decoction, which is in the process of being approved in Mali. The regulatory requirements for herbal medicines are completely different to those for new drugs. It should be emphasized that the primary objective of the project described here was not to develop new drugs, but to improve the utilization of herbal medicines, which are already in use. All the clinical studies described below are reviewed and approved by the Ethics Committee of the Institut National de Recherche en Santé Publique (INRSP) in Mali. This process took six years and cost about 400,000 Euros. The research project is described here as it happened. Some aspects are reviewed in the discussion section, as with the benefit of hindsight some procedures might be improved. The hope is that this paper may help others who

are interested in conducting a similar process (developing phytomedicines, for other indications) through a clear report of what is planned, what is opportunistically added and what is obtained.

Stage 1: Herbal Remedy of Selection

The research project is described here as it happened. Some aspects are reviewed in the discussion section, as with the benefit of hindsight some procedures might be improved. The hope is that this paper may help others who are interested in conducting a similar process (developing phytomedicines for other indications) through a clear report of what is planned, what is opportunistically added, and what is obtained.

In the RTO, the first step is to understand local concepts and terms for diseases. The aim is to maximize the chances that the respondents giving information about the disease of interest to researchers. For uncomplicated malaria, the definition "fever with no other obvious cause during the rainy season" and for severe malaria, it is "fever with convulsions or loss of consciousness during the rainy season". In Mali, these correspond to the local Bambara terms "sou-maya" and "kono" respectively. Of course, these are not very precise, but they are the same definitions as those used for presumptive treatment, and the best that can be done retrospectively when blood tests are impossible.

The second step is to choose a representative random sample of households in the study area (by cluster sampling), and to ask in each whether anyone has had the disease of interest in the recent past. The timing is at the end of the malaria season (for example in Mali the rains begin in July and the perfect time for such a study would be in November-December). For uncomplicated malaria, a recall time of two weeks was considered as this is a common event, and there is a risk of an accurate information during too long recall period. For severe malaria (which is rarer and more dramatic, so more likely to be remembered), a recall period of six months has been used. The sample size is determined based on the estimated prevalence of malaria in the area, and the estimated number of different treatments (from previous information).

The third step, if a respondent had the condition of interest, to ask in detail what treatments they had taken, order of treatment, stage of recovery and whether the cure was complete or with sequelae. Hence, it is possible to understand what treatments patient has been using in real life and with what results.

In Mali, use of this method resulted in a database of treatments taken for malaria cases in 952 households. The analysis is an iterative process performed with the help of a statistician, starting with a test of correlation between reported clinical outcome and the plant used. Since in some cases, recipes contained more than one plant, a second step has been to adjust for this in the analysis, to determine whether individual components were associated with clinical outcomes. From the 66 plants used for the treatment of malaria in the two districts studied in Mali, alone or in various combinations, the one associated with the best outcomes has been a decoction of *A. mexicana*. At this stage, there is the opportunity to evaluate some plants for their anti-malarial activity *in-vitro. A. mexicana* had the best activity *in-vitro*, both for the extracts in polar solvents and the aqueous decoction. The IC50 of the methanol extract was 1.0 µg/mL, which is of the same order as the ethanolic extract of *Artemisia annua*. Before proceeding to clinical studies, it is important to establish that the remedy is safe. WHO guidelines. state that: If the product was traditionally used without demonstrated harm, no specific restrictive regulatory action should be undertaken unless new evidence demands a revised risk-benefit assessment. WHO maintains no requirement for pre-clinical toxicity testing; rather that evidence of traditional use or recent clinical experience is sufficient. Indeed often the same plants are traditionally used both as a food and as a medicine and no toxicological tests are required for foods, usually consumed in greater quantities than medicines. Pre-clinical toxicity testing is only required for new medicinal herbal products which contain herbs with no traditional history of use. Therefore, if preliminary field studies (such as the RTO study) have shown that the preparation is of common and ancient use, with no known important side effects, toxicological studies are unnecessary.

The literature has searched extensively to see whether the safety of the remedy had already been established in previous studies. The aim is to find studies of the same plant part, using a similar extraction method, to answer the following questions:

Are there any reports of human toxicity associated with ingestion of the plant? If so, which part of the plant, in what preparation, at what dose, and what were the consequences? Have any laboratory studies of toxicity been conducted on the relevant preparation of the plant? If so, what did the results show?

What pharmacologically active compounds does this plant species contain? In which parts of the plant are they found? What are their principle pharmacological effects, and at what doses?

Search terms included the plant species and major chemical compounds known to exist in the plant. None

of the existing databases or books can cover all published information on a given topic, and therefore as many sources of information as possible were consulted: firstly freely available online databases then reference books such as pharmacopoeia and similar monographs and texts on plant toxicology and herbal medicine safety and finally, other databases: EMBASE, CAB Global Health, and the Allied and Complementary Medicine database. In the case of *A. mexicana*, the literature search revealed no toxicology studies but there were reports of "epidemic dropsy" in India attributed to the ingestion of the seed oil containing sanguinarine, as a contaminant in culinary oils This was of some concern, therefore the traditional healer was asked to remove seed capsules from the preparation used for clinical studies in Mali. However, there were no references to toxicity from an aqueous decoction of the leaves and stems (which was the traditional preparation in question), and this remedy was reported in the ethno- botanical literature as being use in Benin, Mali, India and Colombia.

Stage 2: Dose-escalating Clinical Study

As patients has been using the remedy in any case, and the literature search did not reveal any concerns, an observational clinical study has been organized with a small number of patients. It is a prerequisite to conduct such a study in an area where patients are already taking the remedy, so that one is not proposing a new treatment (for example for a comparative prospective study – see below) without some clinical evidence of effect size and safety.

The traditional preparation was given to patients with uncomplicated malaria, who met the following criteria:

Inclusion Criteria

- Symptoms of malaria (fever) within the last 24 hours
- Parasitaemia >2000/mcl and <200,000/mcl on microscopy
- Informed consent of patient or parent

Exclusion criteria:

- Signs of severe malaria
- Age <3months
- Pregnancy
- Other concomitant febrile illness administration of a full course of anti-malarial (modern or traditional) within the previous week inability to return for follow-up.
- Patients were followed up closely on days 1, 2, 3, 7, 14 and 28, and were advised to return immediately at any other time if their condition deteriorated.
- Monitoring included parameters of efficacy (temperature, symptoms, parasite counts) and safety (new symptoms/adverse events, ECGs, and blood tests to monitor bone marrow function, renal function and liver function).
- The design of the whole study became a sequential follow-up of patients using, in the first group, a dose lower than the one traditionally used (but, at the time, proposed as the correct one by the traditional healer), then the bottom and top of the usual dose range.

In this way patients always received the best dose according to the current state of knowledge. If the incidence of important adverse effects reached an unacceptable level, the trial could of course be stopped. Compliance was monitored by direct observation of some doses of the treatment (the first dose of each of the first three days when the patient attended for follow-up) and by asking patients whether they had taken the recommended dosage during rest of the day. Thus, it was also possible to assess whether the optimal dosage was realistic and feasible in the field. The outcome measures chosen were appropriate to the context in which use of the phytomedicine was envisaged, which was a high-transmission areas. In low transmission areas, the outcome recommended by WHO is "Adequate Clinical and Parasitological Response" (ACPR) which includes a requirement that the parasite count by day 3 is reduced to <25% of baseline, and that total parasite clearance is achieved by day 7 and maintained through till day 28. Although WHO now also recommends total parasite clearance in all situations, this may not be necessary in high trans- mission areas where the population develops partial immunity in early life, and is rapidly re-infected even if parasite clearance is achieved. In such high transmission areas, the most useful outcome measures are clinical rather than parasitological. One such is the rate of "adequate clinical response" (ACR, see table 3), which is a modification of ACPR. The criterion that parasitaemia on day 3 should have decreased to <25% of that on day 0 was designed for fast-acting drugs, such as chloro- quine and artemisinin derivatives. It is not an essential criterion for testing slower-acting drugs, such as quinine and herbal remedies, and therefore can be omitted for such trials. If patients clinically worsened or did not recover (i.e., "treatment failures") they were given an alternative treatment (the nationally recommended anti- malarial).

Stage 3: Randomized Controlled Trial (RCT)

As results from all previous stages are encouraging, the aim at this stage is to evaluate the effectiveness of the phytomedicine in the field. In Mali, the objective is to develop a phytomedicine for home-based management of malaria (HMM), with the aims of symptomatic

improvement and preventing severe malaria. The vision is that, if effective, the plant could be recommended to communities to be cultivated and prepared locally as a first-line treatment for presumed malaria. Therefore, the inclusion criteria for the RCT reflected this: all patients with presumed malaria (history of fever during the last 24 hours, without another obvious cause, during the rainy season).

Stage 4: Isolation and Testing of Active Compounds

This is the last step of "reverse pharmacology". A phytomedicine can be developed without isolating an active ingredient, but it is useful to do this for two reasons. Primarily, there needs to be a phytochemical marker for quality control and standardization of the herbal medicine, and also to permit agronomic selection of the best plants. Secondly it is possible that a new modern drug could be developed in parallel by the pharmaceutical industry. However, it makes more sense to do this after the clinical safety and effectiveness have already been demonstrated, as chances may be higher that the isolated compound (or a derivative) will also be safe and effective. Much time and money are wasted in developing drugs which turn out to be unsafe or ineffective in humans.

Isolating pure active ingredients from a phytomedicine is not straightforward. Most phytomedicines contain several compounds with additive or synergistic activities, or even pro-drugs. *A. mexicana* contains at least three protoberberine alkaloids in similar amounts (around 0.5% in the plants from Mali) with similar anti-malarial activity: berberine, protopine and allocryptopine (IC50 *in-vitro* = 0.32, 0.32, and 1.46 mcg/mL, respectively). Whereas all are active *in-vitro*, the absorption of berberine is poor in some animal models, although it can be improved by P-glycoprotein inhibitors. It is not known whether *A. mexicana* contains any P-glycoprotein inhibitors, but if it does, their concentration would also be important. The pharmacokinetics of protopine and allocryptopine have not yet been studied in humans, so it is not known which of these is the best marker, or whether there is cooperation between them (in which case maybe all should be used as markers). Unlike berberine, protopine and allocryptopine show a good selectivity (both in mouse and in rat models *Plasmodium berghei* and *Plasmodium chabaudi,* respectively, unpublished results), the plan for *Plasmodium* and their cytotoxicities are low Since preliminary *in-vivo* tests using freeze dried AM decoction were unsuccessful is now to study the *in-vitro* antiplasmodial activity of plasma samples from healthy volunteers to identify plant substances or metabolites involved in such activity.

DISCUSSION

While developing new compounds from natural products could be an important source of new anti-malarials in the long term, it is also possible to develop standardized and validated phytomedicines more quickly and cheaply. The scheme used has already saved considerable time and money in developing a new herbal anti-malarial in Mali. Since the studied plant is a pan-tropical weed, results of such a research programme could be applied in many countries, provided there is local quality control of the plants.

It is of paramount importance to conduct such research in an ethical manner, and all the clinical trials were submitted and approved by an ethics committee. To be ethical, a non-inferiority trial needs to evaluate a strategy that could be sustained after the end of the research. During the study there must be proper safeguards in place to ensure the safety of the patients, so a medical team was stationed in the village for the whole period to give immediate care when required. The result was to inform the villagers which of their traditional remedies has been clinically shown to be an effective anti-malarial, at what dose, what precautions are necessary in its preparation, and that they should rapidly seek modern medical treatment if they do not improve.

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