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Domestication of *Artemisia annua* Plant and Development of New Antimalarial Drug Arteether in India*

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Malaria is one of the world's most devastating human infectious disease and, in India, it is endemic with the mortality rate steadily increasing. As a result the search for the development of new and more effective chemotherapeutic agents to control this life threatening disease is going on at several centres. In this context, artemisinin isolated from the Chinese herb qinghao (*Artemisia annua*) and its derivatives such as arteether, artemether, artesunate, and artilinate have been reported to be effective schizontocidal agent. The Central Institute of Medicinal and Aromatic Plants (CIMAP), in association with Central Drug Research Institute (CDRI), Lucknow had taken up the task of development of α/β–arteether derivative of artemisinin for convenient parenteral treatment of severe and complicated cerebral malaria. The epimeric mixture of α and β-arteether has the advantage of higher solubility in oil medium and is more economical for large scale production. *Artemisia annua* plant was introduced in India by CIMAP. It also developed the appropriate agrotechnologies for its cultivation under the temperate and semi-temperate climates. CIMAP developed the improved varieties containing high artemisinin content and also efficient isolation procedures for artemisinin and essential oil. CIMAP has also developed an efficient and economical procedure for the preparation of drug arteether. The drug arteether has been marketed by Themis India Ltd under the trade name E-Mal and is most effective against uncomplicated, severe complicated/cerebral and multi-drug resistant malaria cases.

Introduction

Malaria is a highly prevalent mortality causing tropical disease, known for more than 2000 y in Egypt, India, and China. It affects over 103 endemic countries with a combined population of over 2.5 billion people and causes one to three million deaths every year. Nine out of ten deaths occur due to malaria in African areas, south of Sahara. Malaria is a major obstacle in the development and prosperity of nations.

Malaria continues to be a major health problem in India. The two main strains of malarial parasites prevalent in India are P. vivax and P. falciparum. In some parts of the Indian states like Assam and Bihar, cases suffering from P. malariae have recently been reported. Every year 2.5 million persons suffer from this disease, the most challenging part being a continuous rise in the incidence of P. falciparum malaria. Over the last decade, it is well known that P. falciparum is the most serious form of malaria with a high mortality rate. Added to this is the fact that most of the strains of P. falciparum are multiple drug resistant in Southeast Asia, including India. In 1994-95, the country has witnessed major outbreaks of such drug-resistant P. falciparum malaria in several parts. In India the foci of chloroquine resistance have now spread throughout the country. According to the World Health Reports, resistance to other powerful antimalarial drugs like mefloquine, halofantrine, pyronaridine, quinine, fansimef has been gradually emerging and areas of resistance have been expanding since 1978. Several reports of chloroquine resistant P. vivax have appeared in India, Papua New Guinea, and Myanmar during the last decade.

Cerebral malaria is a leading cause of mortality amongst the malaria affected population and the mortality reaches up to 30-35 per cent in childhood malaria. From the onset of coma the survival of cerebral malaria cases is generally for 72 h. Besides comatose condition, there are several other complications during acute falciparum infection.

Since 1984, global efforts are continuing to develop fast-acting blood schizontocides control of *P. falciparum* infections, particularly for the treatment of major complications of cerebral malaria. Development of a safe gametocide to stop transmission of drug resistant strains of *P. falciparum* has assumed high priority in the malaria control programme. Because of the lack of safe gametocides the foci of drug resistant malaria are ex-

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panding due to persistent vector transmission. Considering the aforesaid problems associated with falciparum malaria, major objective of our studies at CIMAP has been to develop new antimalarial drugs which can tackle the issues of drugs resistance, cerebral malaria and complicated severe malaria cases.

Artemisia annua Plant in Treatment of Malaria

Quinine the first natural drug introduced for malaria, has become the basis for many synthetic analogues, to date. It is obtained from species of Cinchona from south America. A continued search for such natural drugs led to newer sources. Thus the Chinese drug Quinghaosu (Artemisinin) obtained from the herb "QingHao" (*Artemisia annua*: Family:Asteraceae) which is also known as sweet wormwood was broght into light. In ancient Chinese medicine, this drug was used for the treatment of febrile illness.

The antimalarial activity of qinghao was rediscovered by the Chinese scientists in 1971. The crystallized active principle named qinghaosu (artemisinin) was isolated, characterized and defined as possessing antimalarial properties.

Artemisinin is an antimalarial agent, based on cadinene skeleton, a chemical structure radically different from other nitrogenous heterocycles. The compound is a sesquiterpene lactone containing an endoperoxide linkage which is quite unusual in an antimalarial activity. Artemisinin has been found to be active against chloroquine and multi-drug resistant *P. falciparum* malarial parasite, particularly in cerebral malaria with remarkably fast onset of action and fewer side effects.

Life Cycle of the Malarial Parasite and Antimalarial Drugs

The malarial parasites are transmitted from man to man by female anopheles mosquito. Malarial infections begin when infected mosquito bites humans, it injects thin, spindle shaped forms of the parasite, called sporozoites into the blood, which enter liver cells where they grow into tissue schizont for about 7 d to produce over 30,000 daughter individuals or merozoites. These flood into the blood strem and invade red blood cells, feed on Cost Raemoglobin and undergo further multiplication (schizogony). This results in the release of about 15-20 merozoites which invade further RCB cells, a process that is repeated indefinitely causing fever at the end of each schizogony. During the blood phase, some merozoites enter blood cells and develop into male and female sexual stages which are taken up by a mosquito when it feeds on the patients. Within the gut of the mosquito the male and female gametis fuse and a third phase of multiplication occurs on mid gut on mosquito, resulting in the production of thousands of sporozoites. These enter the mosquito's salivary glands to be injected into a new host when it feeds on healthy subject. These gametocytes do not cause any clinical symptoms of malaria but are responsible for further transmission of malaria.The sporozoiters of *P. vivax* and *P. ovale,* in humans, differentiate into pre-erythrocytic tissue schizonts and large number remain dormant to produce. The hypnozoites remain dormant in hepatocytes for considerable periods. At a predetrimined time the hypnozoites begin to grow and undergo exoerythrocytic schizogony forming a wave of merozoites that invade the blood and produce a clinical relapse. There is no evidence of true relapse in *P. falciparum* (since their sporozoites do not differentiate into hypnozoites).

Antimalarial drugs have selective action on the different phases of the Plasmodium life cycle and may be classified as follows:

(i) *Casual Prophylactive Agent* – Prevent the establishment of the parasites in the liver. There is no drug available which is a casual prophylactic at safe dose. *Primaquine* is effective but is less safe.

(ii) *Blood Schizontocidal Drugs* – Attack the parasite in the red blood cell, preventing (clinical prophylexis) or terminating the clinical attack (treatment) when the term Schizonticidal drug is used alone it refers to action on blood schizonoites, e.g., chloroquine, quinine, amodiaquine, mefloquine, sulphadoxine, pyrimetamine, and artemisinin derivatives.

(iii) *Tissue Schizontocidal Drugs* – Refer to compounds like Primaquine acting on hypnozoite/ exoerythrocytes stages. They prevent relapse of *P. vivax* and *P. ovale* malaria by destruction of hypnozoites in the liver.

(iv) *Gametocytocidal Agents* – Destroy the sexual forms of the parasites in the host and prevent further transmission of malaria, e.g., Primaquine which acts on gametis of all four species of Plasmodium, especially *P. falciparum.*

(v) *Sporontocidal Drugs* – Inhibit the development of the oocysts on the stomach wall of the mosquito feeding on the human carrying gametocytes so that no sporozoites are produced and mosquito cannot transmit the infections. To be effective, drug should be available in blood meal, e.g., Primaquine.

Newer Drugs

(i) *Halofantrine* – It is a phenanthrane methanol compound. It is active against multidrug resistant falciparum malaria. There is no parenteral formulation and there are limited published data on Pharmacokinetics and toxicity. The main problem with current formulations of halofantrine is poor oral bioavailability.

(ii) *Pyronaridine* – This Napthyridine derivative was synthesized in China and is highly active against multi-drug resistant *P. falciparum. o*ral bioavailability of Pyronaridine from tablets and capsules is poor.

(iii) *Qingaosu (Artemisinin) Derivatives – Artemisia annua* (Qinghao) is a Chinese herb used for treating malaria. The compounds derived from this herb are most rapidly acting schizontocidal drugs. Various derivatives have been formulated such as artemisinin, artemether, and artesunate. They are effective when given parenterally, orally or by suppository. No serious adverse effect has yet been reported in human.

Artemisinin is perhaps the best known plant derived antimalarial drug after quinine and is now likely to prove the main alternative in the case of chloroquine and quinine resistant malaria. Although some other species of Artemisia display antimalarial activity but none, apart from A. annua, appears to contain artemisinin. Several semisynthetic derivatives of artemisinin have been prepared such as methylether and artesunate (with improved pharmacokinetics and better antimalarial activity). Some attempts have been successfully made to synthesize molecules model upon the unique artemisinin endoperoxide, which is essential for activity. It is hoped that progress in this area could remove reliance upon the plant as a source of the drug. Total chemical synthesis of artemisinin, however, has been found to be economically unviable. Hence the isolation of naturally occurring artemisinin from *A. annua* remains the only source of the drug.

In view of the usefulness of artemisinin and its derivatives to treat malaria clinically, particularly in the Indian context, where drug resistant falciparum malaria is on the rise and deaths occurring owing to cerebral malaria frequently. CIMAP, Lucknow launched the drug development programme for artemisinin and its derivatives, in collaboration with CDRI, Lucknow. The drug development programme includes successful introduction of *A. annua* plant in India, its genetic improvement for higher yields of artemisinin large scale cultivation of the plant, pilot scale isolation of artemisinin, synthesis of potent lipophlic derivative arteether (ethyl ether of dihydroartemisinin) and other derivatives, pre-clinical, antimalarial pharmacological, toxicological, chronic toxicological and formulation studies. The R&D efforts made and results obtained are briefly described.

Introduction of *A. annua* and Development of Agrotechnology in India

The plant *A. annua* is indigenous to China, Southern USSR, Turkey, Iran, Afghanistan and naturalized in the US's. It is a temperate plant and, as such, it requires cold winter and moderate summer. However, it can also be cultivated in subtropical areas as a winter crop like north Indian plains in Lucknow. In India, *A. annua* was introduced by CIMAP in 1986, from the Royal Botanical Gardens, Kew, England and agrotechnology for cultivation of the crop has been developed. In recent years, breeding experiments have led to development of high artemisinin and artemisinic acid yielding genotypes cultivars called Jeevanraksha has been developed whose leaves and flowers contain about 1 per cent artemisinin. *A. annua* has been observed to grown well in light loam soils rich in organic matter. . The plant is propagated through seeds. Seeds can be directly drilled into soil and also seedlings can be raised in nursery and transplanted into the field. Transplanting of seedlings gives better results. The ideal time of transplanting in temperate areas is April and May, while in subtropical areas, the planting time is the month of November. Commercial crops of Jeevanraksha yield about 20 to 40 kg artemisinin/ha, under north Indian plains conditions. The crop is planted at high density of about $2x10^5$ plants/ha for high yield of artemisinin.

Development of Processing Technology for Artemisinin

Air-dried leaves and flowers of *A. annua* cultivated at Lucknow and Srinagar were extracted by petroleum ether in the batch of 50 kg dry plant material. In 1985-86, a total of 3.5 tonnes of herb was extracted. Artemisinin being present in a very small concentration 0.01-0.1 per cent in the plant material was successfully isolated in high purity by developing advanced chemical processing technology at pilot plant scale. The process included the extraction of plant material with *n*-hexane. The hexane extract was concentrated under reduced pressure and the concentrate was defatted by methanol. The methanol soluble portion was concentrated and residue was chromatographed on silica gel. The ratio of residue to silica gel was 1:10 for satisfactory isolation of artemisinin. Elucidation of column by hexane-ethyl acetate mixture afforded artemisinin rich fraction which on concentration and recrystallization yielded pure compound artemisinin (mp 153-154°C).

Regeneration of Silica Gel and Purification of Crude Extract

To economize the isolation procedure further improvements were carried out in the process. Since large amount of silica gel is used in the chromatographic seperation of artemisinin, a process for regeneration of silica gel was developed so that it can be reused again. The regeneration process of silica gel in 100 kg batch size was developed and same silica gel was used three-times with efficacy. It will reduce the cost of isolation of artemisinin.

Simultaneous Production of Artemisinin and Essential Oil from *Artemisia annua*

A. annua the only source of artemisinin, is also a good source of essential oil. Besides China the plant grows wild in many European countries, where it is used for its aromatic oil. The essential oil is used by the flavouring and fragrance industries.

At present, no such process is known where both the components, artemisinin and essential oil can be isolated without destroying either. An improved process has been developed in which artemisinin and essential oil both could be isolated from the same plant material. Isolation of artemisinic acid and artemisinin in this process, without chromatography and reuse of solvents will tremendously reduce the cost of production of artemisinin.

Artemisinic acid obtained in this process is converted into artemisinin, which will increase the over all yield from the plants by more than double.

Semi Synthesis of Artemisinin from Biogenetic Precursors

Conversion of Artemisinic Acid and Arteannuin B into Artemisinin

The occurrence of artemisinin in the plant of *A. annua* in low yield (0.01-0.2 per cent) and the fact that none of the reported chemical synthesis is economically viable, efforts have been made to convert abundantly present artemisinic acid and *arteannuin B* to artemisinin. Due to high concentration of artemisinic acid, attempts were made by many workers to convert the artemisinic acid to artemisinin. An improved process has been developed in which reaction can be carried out at room temperature and no chromatography process is involved for the purification of compound. The process has resulted in increased yields (40 per cent) of artemisinin by photooxidation of dihydroartemisinic acid. The improved process provides a simple, less expensive, and more efficient process for production of artemisinin from the plant material. Thus the yield of artemisinin is increased two- to three-fold.

Arteannuin B (*artemisinin B*) is another constituent of *A. annua* which is available 2-3-times more the amount of artemisinin. There is only one procedure reported for the conversion of *arteannuin B* into artemisinin. Attempts were therefore made to devise a cheaper method for above conversion.

Arteannuin B was reduced with Nickle Iboride to obtain 90 per cent yield of *dihydroarteannuin B*. Reaction of dihydro *arteannuin B* with BF_3- acetic anhydride was carried out with the aim of obtaining the allylic acetate for eventual formation of the *deoxydihydroarteannuin B* by reductive elimination of the acetate function. However, it was observed that allylic acetate is the minor reaction product and the major product was identified as the deep seated rearranged lactone. This compound was characterized by spectral studies, chemical transformations, and finally by single crystal X-ray analysis of the corresponding alcohol.This alcohol opens up new vistas for the preparation of 9,10 substituted artemisinin analogue by simple chemical manipulations.

Synthesis and Upscaling Process for Arteether

Artemisinin is sparingly soluble in water or oils and not well absorbed by gastrointestinal trac. A search for more potent analogues of artemisinin with better bioavailablity was therefore made. WHO group on chemotherapy of malaria decided in 1981 to accord high priority to the improved the water solubility of ar-

temisinin. A water soluble derivative artesunate, was prepared which was a fast-acting compound and appeared after greater life saving potential than even chloroquine, or quinine, for the treatment of complicated *P. falciparum* cases. However, this compound was found to be unstable in solution. The WHO group, therefore, proposed the development of an oil soluble methyl ether derivative of dihydroartemisinin namely artemether as a new drug for treatment of complicated falciparum cases. However, there is a possibility that this compound could produce methanol after cleavage of ether linkage, resulting in the formation of methanol, formaldehyde and formic acid. WHO shifted their priority since 1986 to develop oil soluble β–arteether (ethyl ether derivative of dihydroartemisinin) as a new drug for emergency treatment of cerebral malaria cases. Among all the derivatives of artemisinin investigated, α/β arteether (30:70 isomeric mixture) was found to be superior to other derivatives because of its better lipophilicity, pharmacokinetic properties, and considerably less toxicity. Arteether, therefore, was chosen as potent candidate drug for clinical trials after successful, preclinical antimalarial, pharmacological, and toxicological studies carried out at CDRI.

Synthesis of Arteether

Arteether is prepared in two steps using batches of 10 g artemisinin. The first step in the preparation of arteether involves reduction of artemisinin with sodium borohydride into dihydroartemisinin and subsequent etherefication of dihydroartemisinin by lewis acid catalysed reaction, affording an epimeric (80:20 mixture of β and α isomer) ether of dihydroartemisinin. Both the epimers were separated by column chromatography and crystallized to yield crystalline β-arteether and oily α-arteether. The structures were determined by spectroscopy techniques. A 30:70 epimeric mixture of α- and β-arteether was taken as the antimalarial drug. During clinical trials as per requirement CIMAP has prepared arteethers, purified and separated it into α- and β-arteether. Pharmaceutical grade of α/β-arteether (30:70) was formulated and supplied to CDRI for clinical trials.

Stereoselective Preparation of α-Arteether

A new and efficient process for the preparation of α-ethyl ether of dihydroartemisinin has been developed. The process gives an easy access to α-arteether only, which being oil itself is easily soluble in refined groundnut oil, the vehicle for intramuscular injection.

Epimerization of β-arteether into α-Arteether

Both the isomers of arteether are equally effective. α-arteether has advantage over β-arteether, particularly with regards to higher solubility in lipids. Conversion of β-isomer to α-isomers is a tedious operation that we have achieved with anhydrous $FeCl_3$ under specific reaction conditions.

An Improved Procedure for the Synthesis of Arteether

Artemisinin and dihydroartemisinin are quite unstable molecules under acidic conditions for the preparation of arteether. Only two methods have so far been reported and both are using stringent reaction conditions. Brossi *et al.* have used BF_3-Et_2O at 70°C and El-feraly *et al.* use a two step reaction using P-TsOH as an acidic catalyst. We have developed a simple and efficent procedure which is of general application for the synthesis of ethers of dihydroartemisinin using chlorotrimethylsilane as catalyst. Ether derivatives were prepared in 85-90 per cent yield. Other advantage of this method is that the reaction is carried out at room temperature without nitrogen atmosphere and 30:70 ratio of α:β-isomer of ethyl ether derivative which we are developing as a new drug, is obtained from the reaction mixture. Artelinic acid is most active and stable water soluble derivative being developed as a new drug was prepared by this procedure.

Estimation of Artemisinin in *Artemisia annua*

For the estimation of artemisinin content in plant sample, a faster, TLC scanning procedure was developed. The dried plant material was powdered, extracted with *n*-hexane and concentrated. The residue was dissolved in EtOH and chromatographed on TLC plate using *n*-hexane-ethyl acetate (85:15) as solvent and develop the plate in *p*-dimethylaminobenzaldehyde solvent colour reagent. The plate was heated at 110°C in oven to develop colour. The plates were scanned at 445 nm for measurement of artemisinin.

Estimation of α- and β-isomer of Arteether

TLC densitometric estimation method for α- and β-isomers of arteether samples were developed. The assay combines absorption of arteether on siglica gel 60 F_{254} plate and TLC spot visualization by spraying plates with *p*-dimethyl-amino benzaldehyde (0.5 per cent solution in MeOH containg 1 per cent conc. HCl) and estimation by dual wavelength absorption and TLC plate were scanned at 580 nm and 710 nm.

Estimation of Artemisinin, Artemisinic Acid and Arteannuin B

A rapid, simple, and sensitive reverse phase high performance liquid chromatography (HPLC) method for the simultaneous estimation of three important sesquiterpenes of *A. annua* plant was developed. Use of two different reverse phase columns ODS and CN in series resulted in a good resolution with maximum recovery of three sesquiterpenes artemisinin, arteannuin B and artemisinic acid when water with 1 per cent TFA-acetonetrile (30:70) was used as mobile phase.

Antimalarial Activity of Artemisinin and Arteether

Antimalarial activity of artemisinin (Qinghaosu) and arteether against blood induced *Plasmodium berghei* infection was evaluated in Swiss mice. Artemisinin, in doses, ranging from 3.12 to 100 mg/kg (administered intramuscularly by as an aqueous suspension) for 4 d did not have any suppressive effect. When it was given in neutralized ground nut oil (Arachis oil), a dose of 100 mg x 7 d was fully curative. Arteether showed 80 per cent curative action when single or two doses of 5 mg/kg drug were given in oil intramuscularly. Three dose treatment at 5 mg/kg was fully curative against sensitive strain of *P. berghei*.

Blood schizontocidal activity of epimers of arteether (α-, β-arteether and α/β-arteether (30:70) has been evaluated against a multi drug-resistant strain of *P. yoelii nigeriensis* in weanling rats. Both the drugs were dissolved in sterile groundnut oil and administered intramuscularly. α/β-arteether has shown curative action at 2.5 mg/kg x 3 doses in rats while arteether was consistently curative against above infection at 5 mg/kg x 3 doses. Arteether was found to have curative action at 5 mg/kg x 3 d against blood-induced *P. cynomogi B* infection in rhesus monkeys, while the curative dose of artemisinin oil suspension was 10 mg/kg x 7 d or 20 mg/kg x 3 d Parasite clearance with arteether was achieved within 24 h. Neither arteether nor artemisinin exhibited any casual prophylactic or radical curative action up to a dose of 20 mg/kg against sporozoite- induced *P. cynomolgi B* infection in rhesus monkeys. A multi-drug resistant strain of *P. yoelii nigerensis*, resistant to chloroquine acid mefloquine (128 mg/kg x 4 d), and quinine (400 mg/kg x 4 d) was found to be completely susceptible to arteether at a dose of 5 mg/kg x 3 d administered intramuscularly. Artemisinin at 50 mg/kg x 7 d had only suppressive action against this strain.

Gametocytocidal Effect of Arteether to Block Malaria Transmission

The increasing incidence of *P. falciparum* and expansion of foci of chloroquine resistant strains is causing unprecedented threat for the malaria control program in most of the tropical countries including India. The only gametocytocidal drug available for interrupting *P. falciparum* transmission is primaquine, developed 50 y ago but because of its inherent toxicity and side effects the use of this drug is restricted. Global efforts are continuing to replace primaquine with a safe gametocytocide. Besides the blood schizontocidal activity, arteether has also shown strong gametocytocidal activity against *P. cynomolgi. B*. A stephensis model and the drug has been found to completely sterilize the mosquito infectivity as evidenced by absence of oocyst development on the midgut of mosquitoes fed on drug treated gametocyte carrying rhesus monkeys. A single dose of 5 mg/kg arteether given by intramuscular route as well as 5-10 mg/kg by oral route produced 100 per cent inhibition of mosquito infectivity. This drug has strong capacity to stop transmission of malaria by virtue of its gametocytocidal effect.

Antimalarial efficacy of α/β-arteether, particularly the blood schizontocidal activity has been tested in two species each of malaria in mice and rhesus monkey. The initial test indicated antimalarial efficacy of arteether against blood stages of *P. berghei*. The drug was found to be curative at 5 mg/kg x 3 d. The strain of *P. yoelii nigeriensis* used in this study showed resistance to oral administration of mefloquine, quinine, and chloroquine and a minimum dose 5 mg/kg x 3 d (intramuscular) in neutralized oil was curative and recurredescence of parasitaemia was not observed. Further studies showed that curative dose of arteether against *P. yoelii nigeriensis* in albino rats was 2.5 mg/kg x 3 d. Further studies showed that dose of 5 mg/kg x 3 d was fully curative against blood induced *P. cynomolgi B* infection. Arteether is curative at 12 mg/kg x 3 d against acute infection with *P. knowlesi* in rhesus monkeys, which were in precoma stage. The drug was curative even at higher parasitaemia ranging from 10 to 30 per cent. These results suggested that arteether is effective in controlling acute malaria infections and a potent drug for the treatment of cerebral malaria. Which is supported by its curative action against both *P. fragile* and *P. knowles*.

Animal Pharmacological Studies on Artemisinin

Pharmacological studies on artemisinin have been carried out in experimental models by giving the drug as

oil suspension intramuscularly. Doses ranging from 30-200 mg/kg of artemisinin were devoid of significant pharmacological effects on central nervous system, cardiovascular system and urinary system. The drug has not shown any anti-inflammatory or anti-allergic activity. No adverse pharmacological effects have been observed in this study.

Pharmacological Studies on α/β-Arteether

The drug is given to experimental animals in neutralized arachis oil suspension. The studies include recordings of changes in gross behavioural effects at 215-1000 mg/kg dose, analgesic activity at 20 mg/kg, anti-passive cutareous anphylactic activity at 50-100 mg/kg dose in mice, changes in blood pressure, respiration, ECG and nictitating membrane contraction in anaesthetized cats at 3-3.5 mg/kg dose, diuretic activity at 100 mg/kg and anti-inflammatory activity at 50 mg/kg dose in rats. These doses of arteether were devoid of any significant pharmacological effects on central nervous system, cardiovascular system, and urinary system. Further the drug had no anti-inflammatory response or anti-allergic activity, through it demonstrated some anti-anaphylactic activity at higher doses.

Sub-acute Animal Toxicity Studies – The toxicity studies of the compound α/β-arteether were carried out in charles foster rats (*Rattus rattus*) and rhesus (*Mucaca mulatta*).

Acute Toxicity – The LD_{50} in mice was >1000 mg/kg (im).

Sub-acute Toxicity – The results of sub-acute toxicity study conducted in rats and monkeys are as follows.

Rats – Toxicity test consisting of daily intramuscular injections of arteether in arachis oil, three consecutive days a week for 4-weeks, in doses of 2.5, 5.0 and 10.0 mg/kg body weight in different groups of rats did not show any adverse effect on the animals. The controls were injected with comparable volumes of sterile arachis oil alone. Experiment was terminated by sacrificing the animals two weeks after the last course of injections. Findings of haematology, biochemistry, and histology were found to be well within the range of normal.

Monkeys – Toxicity tests consisting of daily intramuscular injections of arteether in arachis oil, three consecutive days a week for 4-d, in doses of 7.5, 15 and 30 mg/kg body weight in different groups of rhesus monkeys showed no drug related adverse effect on the animals. The controls were injected with comparable volumes of arachis oil alone. The experiment was terminated by sacrificing the animals two weeks after the last course of injections. Findings of haematology, biochemistry, and histology were found to be well within the range of normal. Arteether has been found safe in rats and rhesus monkeys in the doses mentioned above. Hence, it is concluded from our experiments that arteether is safe in rodents and non-human primates and can be used for clinical trials in humans.

Clinical Studies with α/β-Arteether

Phase I single and multiple dose tolerance studies have been undertaken at CDRI with the aim of evaluating tolerability of α/β-arteether after single and repeated dose administration. Phase I single dose tolerance study was conducted in 30 healthy human volunteers. The trial was double blind, placebo controlled, and non-cross over. The dose of arteether injections ranged from 20, 50, 100, 150, 200,and 300 mg in 2 ml arachis oil, and identical ampoules containing arachis oil as placebo were used . Each group had three volunteers receiving one dose one volunteer receiving placebo and one receiving next higher dose of α/β-arteether in a randomissed double blind schedule. Six volunteers in placebogroup and four each in 20, 40, 399 mg dose group, respectively. Multiple dose study was completed in 20 healthy human volunteers, 10 subjects receiving 100 mg injection and 10 subjects receiving 150 mg injection of arteether, once a day as intramuscular injections on three consecutive days. A detailed pre- and post-drug monitoring of clinical, haematological, and biochemical laboratory investigations did not suggest any drug-related abnormality. Single, as well as repeated, intramuscular injections of arteether/placebo was tolerated well but no tenderness, swelling or discomfort, was experienced by any subject at the site of the injection.

Phase II Clinical Studies of Arteether

To evaluate the effectiveness of arteether, in patients with acute falciparum malaria, arteether was administered to 51 patients with plasmodium falciparum malaria according the dose of 150 mg intramuscularly once a day on three consecutive days. Patients with severe complications like cerebral malaria or senal failure, pregnant woman and those who had already taken antimalarial drugs were excluded. Complete parasite clearance

from the peripheral blood was observed in 80 per cent of the patients at 48 h and in 98 per cent at 72 h. The medium parasite clearance time was 2 d (range 1-4 d) 65 per cent of the patients became afebrile with in 48 h and 81 per cent by 72 h. The mean fever clearance time was 52.04 h reduction of the spleen size was observed in 70 per cent of all patients in less than 7 d no side effects were seen. Patients were followed up for 28 d, seven were readmitted with *P. falciparum* infection but it could not be ascertained, whether these cases were reinfections or recrudescences. This study thus establishes the efficacy of three days dosage/schedule of arteether given as a single dose of 150 mg without resulting in any side effects or toxicity.

Phase III multicentric clinical trial with arteether were conducted at eight centers in Bhilai, Delhi, Dibrugarh, Guwahati, Jabalpur, Jasmeshedpur, Rourkela and Sonapur, in 267 patients of uncomplicated and 211 patients of complicated *P. falciparum* malaria. Each patient was treated with a fixed dose schedule of arteether given intramuscularly in a dose of 150 mg once a day for three consecutive days. Tolerability of arteether injection was good in all the cases and no significant adverse drug reactions were encountered during the trial. In uncomplicated group fever clearance time ranged of 24-68 h, parasite clearance time was recorded in the range of 24-72 h and 97 per cent cure rate was observed. The recrudescence reinfection rate of 3 per cent was observed in this study. In complicated group fever clearance time ranged between 24-168 h, parasite clearance time ranged between 24-120 h and overall mortality in this group ranged between 4.8-5 per cent, (14 out of 211 patients) of these, 10 patients expired within first 2 d before completing the 3 d schedule of arteether therapy and cure rate in this group ranged between 91.5-100 per cent. These phase III multicentric clinical trials in 478 patients of *P. falciparum* malaria (267 uncomplicated and 211 complicated) have established the efficacy of 3 d schedule of parenteral therapy with arteether over all recrudescence found in these studies was 5 per cent . These clinical trials with arteether have been undertaken as per regulatory guidelines from Drug Controller, General India and have spent over 7 y (1989-96). Recently, it has been cleared for marketing in India in hospital/medical colleges/nursing home, etc.

Distinguishing Features of α/β-Arteether

Arteether shows rapid schizontocidal action with quicker parasite clearance rate and short fever clearance time, virtually with no side effects and low recrudescence rate.

Arteether is lipophilic and have the advantage of greater accumulation in brain tissues, hence a potential drug for the treatment of cerebral malaria.

Arteether is an ideal antimalarial drug, especially for treating drug resistant and complicated *P. falciparum* malaria.

Arteether injection is the only artemisinin derivative which has undergone extensive preclinical, animal and toxicological studies, as well as phase I, phase II and Phase III clinical studies in Indian subjects as per drug regulatory requirements.

Arteether is totally new drug introduced in India for the first time and chance of developing drug resistance and cross resistance with other drugs available in the market is nil.

The speed of action, determined by rapid PCT, efficacy assessed by FCT, low recrudescence rate and virtually no adverse effects makes arteether an attractive alternative to quinine and chloroquine as quinine/chloroquine has got several limitation for its use.

Summary

Artemisia annua a Chinese herb has remained as a potential medicinal plant since ages. The plant has received enormous attention as the only potent source of artemisinin. The potential market for artemisinin and derivatives has promoted the establishment of research programmes of *A. annua* as a commercial crop in India. CIMAP introduced *A. annua* plant in Kashmir and Lucknow climatic conditions. It has now developed high artemisinin yielding lines of *A. annua*, agrotechnology, processing technology for the large scale isolation of artemisinin and other bioprecursors. Artemisinin was converted into arteether and evaluated for antimalarial activity which after completing phase III clinical trials in collaboration with CDRI, Lucknow, is now available in the market in the form of drug. This process technology has outstanding, multi-disciplinary, innovative technological contributions with visible, economic, industrial or social impact.

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