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As pure as possible: India



GENERAL INFORMATION

- ◆ **Implementing institution**
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- ◆ **Head**
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- ◆ **Implementation period**
Two years (1997-1999)
- ◆ **Costs**
Contribution from private sponsoring agency: US\$20,250; contribution from the RRL-Jorhat (CSIR): US\$20,000. Total: US\$40,250.

SUMMARY

The World Health Organization (WHO) estimates that more than 40 per cent of the world population is exposed to the risk of malaria, and more than 3.5 million people die of the disease each year. Resistance of the malaria parasites to chemotherapeutic agents such as chloroquine has increased in recent years and there is an urgent need for new therapeutic agents to be developed.

Artemisinin, a once-promising anti-malarial agent derived from the Chinese medicinal plant *Artemisia annua*, is associated with several drawbacks and is no longer used clinically. However, ether derivatives of dihydro-artemisinin such as arteether and artemether are much better drugs than artemisinin. Of these, arteether is preferred because artemether has been associated with some neurotoxic effects.

The synthesis of arteether from artemisinin results in a mixture of the corresponding α and β isomers, of which the crystalline β -isomer is the actual active drug. As these isomers are difficult to separate, WHO recommends a 40:60 mixture of α and β isomers for clinical use. However, RRL-Jorhat has developed an efficient and environmentally friendly process for producing clinically pure β -arteether. The process has been sold to a private party for commercial production. To date, no other party has commercialized clinically pure β -arteether.

BACKGROUND AND JUSTIFICATION

The seven northeastern States of India, which cover the southeastern sub-Himalayan region, are endowed with vast resources of flora and fauna. Indeed, the region is located within the Indo-Burma "biodiversity hotspot" and is home to approximately half of the total flora of the Indian subcontinent.

Located in this region, the Regional Research Laboratory (RRL), Jorhat, has emphasized the effective utilization of the natural resources of the area since its inception in 1962. Until the late 1980s, however, these activities were mainly academic in nature and resulted in the isolation of more than 150 new and diverse plant secondary metabolites and more than 100 publications in internationally renowned journals.

In the early 1990s, the emphasis shifted towards the extraction and commercialization of drugs and drug intermediates from the region's plants. Since 1997, RRL-Jorhat has been engaged in a major inter-CSIR laboratory programme, "Development and commercialization of bioactive molecules and traditional preparations from medicinal plants". This programme uses the combination of Indian biodiversity and the traditional system of medicine as a source of many bioactive preparations and lead compounds. Already this programme has produced exciting results and several extracts with excellent bioactivity against different diseases have been identified.

In the course of this work, RRL-Jorhat scientists cultivated an Indian variety of *Artemisia annua* (family Asteraceae) and established an extraction procedure for artemisinin, a potent antimalarial drug.

Artemisinin is an unusual sesquiterpene lactone containing an endoperoxide function. Its unique chemical structure, coupled with its low toxicity and proven antimalarial activity, has attracted attention from both chemists and pharmacologists since its discovery in China in the early 1970s. The practical use of artemisinin as an antimalarial drug was impaired, however, by:

- its low solubility in water and oil;
- its poor efficacy by oral administration;
- its short half-life in the blood; and
- the high rate of recrudescence in treated patients.

Studies on the structure-activity relationship of artemisinin have produced several derivatives with improved solubility and efficacy. Among these are artemether and arteether, which are already approved as antimalarial drugs in China.

Despite the availability of effective antimalarial agents, a major factor that has led to the persistence of malaria is the emergence of *Plasmodium* strains that are resistant to one or more classes of antimalarial drug. This resistance is thought to develop as a result of spontaneous chromosomal point mutations, possibly even independently of drug pressure. Subsequently, more resistant mutants are

selected under drug pressure.

India is a high-risk region and urgently needs new drugs for treating patients infected with resistant strains of malaria parasites as well as people suffering from cerebral malaria, the most deadly form of the disease.

As RRL-Jorhat scientists had experience in working with *A. annua* and artemisinin, several Indian companies approached the institute, asking for commercially viable and world-class technology to produce an effective antimalarial drug. RRL scientists have since developed novel laboratory-scale technologies for producing β -arteether from artemisinin. In the RRL-Jorhat process, the desired β -isomer of arteether is obtained in more than 78 per cent yield of the clinically pure product. Recognizing the commercial possibilities of the institute's know-how, a private company, M/s FDC Ltd., Mumbai, came forward to sponsor the scaling-up of the technology. This process has been successfully completed and the technology has been transferred to the commercial sponsor.

DESCRIPTION

To combat the rapid spread of drug-resistant malaria, effective therapeutic agents are continuously being sought, especially against strains resistant to conventional quinoline- and acridine-based drugs. Chinese scientists had already identified artemisinin, derived from *Artemisia annua*, as being superior to con-

ventional antimalarial drugs, such as chloroquine and quinine, against resistant strains of malaria without obvious adverse effects in patients.

As the northeastern region of India is adjacent to China and has similar climatic conditions, RRL-Jorhat initiated a programme in the 1980s to investigate local indigenous plants of the Asteraceae (sunflower) family in general and different *Artemisia* species in particular, looking for artemisinin-type molecules.

During this study, a series of cadinine-type sesquiterpenes were isolated from the plant *Eupatorium trapezoideum* that was structurally similar to artemisinin. One sesquiterpene, in particular, was present at high concentrations. By modifying this molecule, a few artemisinin-type compounds were developed that exhibited moderate antimalarial activity in *in vitro* tests.

Chemical investigation of other *Artemisia* species, including *A. caruifolia* and *A. mantima*, however, did not yield artemisinin-type molecules. Therefore, having failed to identify an alternative source of artemisinin or similarly active molecules, it was decided to cultivate *A. annua* in the region and evaluate its artemisinin content.

A. annua seeds were collected from the Indian Agricultural Research Institute, Pusa Campus, New Delhi, and cultivated at the RRL-Jorhat experimental farm. The plants grew well, attaining a height of about 1.5 metres. However, the maximum content of artemisinin obtained from three different crops was

only around 0.08 to 0.1 per cent dry matter, with the highest content being reached about two weeks before flowering.

To overcome this, cultivation was attempted in other parts of northeast India with different climatic conditions, but yields of artemisinin were still relatively low and even carefully grown plants can be devoid of artemisinin. Plants grown at the RRL branch laboratory at Itanagar, Arunachal Pradesh, for example, yielded only a negligible amount of artemisinin. The best results were obtained in plantations in North Viet Nam, mainly in the vicinity of Hanoi.

A search was therefore made for easily available and inexpensive raw materials. When the project started, the cost of pure crystalline artemisinin in India was high (around US\$1,500 per kilogramme), but a source was identified in China selling pure artemisinin at US\$380 per kilogramme. This was an important good boost for the project, and several kilogrammes were procured.

Starting with pure artemisinin, 5 gramme-scale technologies were developed for producing derivatives such as arteether, artemether and sodium artesunate. At this stage, RRL-Jorhat was approached by a Mumbai-based private pharmaceutical company for a technology to produce arteether starting with 200 gramme batch sizes. The reaction scheme developed involved two steps:

1. Reduction of artemisinin to dihydroartemisinin by sodium borohydride in methanol at -5°C .

Parameters such as substrate dilution,

reagent concentration, reaction temperature, and pH of the reaction mixture are critical for obtaining high yields of purified reduced product. The method was optimized in terms of all these parameters to obtain a 97-per cent yield, which compared well with the 79 per cent reported using other methods. Scaling up the reaction, however, produced unforeseen difficulties. Reduction of artemisinin using sodium borohydride must be carried out at very low dilutions, which requires large volumes of methanol. If the reaction was performed in a more concentrated methanol solution, the yield of dihydroartemisinin was reduced and several unwanted by-products formed. In addition, the reaction temperature had to be maintained between -5° to $+5^{\circ}\text{C}$. Sodium borohydride reduction is an exothermic (heat-releasing) reaction and hence, in the large-scale production of artemisinin, the addition of sodium borohydride to methanolic artemisinin solution must be carefully monitored. Increase of the reaction temperature beyond $+5^{\circ}\text{C}$ has a negative effect on yield. Another major concern faced in this step is the recovery of the spent methanol, which would otherwise add to production costs and disposal problems. Normally methanol would be recovered by fractional distillation, but methanol from this reaction contained a large amount of boron-containing products derived from sodium borohydride

that co-distilled with the methanol, making it unsuitable for reuse. At the time, the commercial sponsor did not press for the recovery of the spent methanol as it is a relatively inexpensive solvent (less than US\$1 per litre) in comparison to the high-value product β -arteether. However, this problem has since been successfully resolved.

2. Etherification of dihydroartemisinin with ethanol in the presence of an acid catalyst.

In this step, normally a mixture of α and β isomers of the ethyl ether derivative of dihydroartemisinin, arteether, is formed, of which the β isomer is crystalline in nature and the α isomer is a gum. As the β isomer is the actual active drug, reaction parameters had to be carefully optimized to yield a favourable proportion of the β isomer compared to the undesired α isomer, especially when the process was scaled up. It was also observed that during the etherification of dihydroartemisinin with ethanol in presence of an acid catalyst, the reaction never reached completion and a small amount of dihydroartemisinin persisted. In small, laboratory-scale batches, this contaminant could easily be separated. However, this posed a problem at the larger pilot scale, which has been overcome by adding an alkali wash to the arteether solution. The dihydroartemisinin, being more hydrophobic and more sensitive to alkali than arteether, is decom-

posed and can be washed away. The addition of this step allows the production of β -arteether with a purity of greater than 98 per cent, separated by crystallization, as desired in the sponsor's product specification (fig.1).

PATENTING AND COMMERCIALIZATION

An Indian patent has been granted for the process: An ecofriendly and economical process for the synthesis of arteether, artemether, etc., from commercial artemisinin. N.C. Barua, M.S. Bezbarua and J.C. Sarma: Indian Patent NF/125/00.

A quick analysis of the production costs of β -arteether reveals that, with a production cycle of three days per batch, up to 250 kilogrammes of the purified product can be produced each year. Taking into account the cost of the raw materials for such a production run (but excluding capital expenditure, labour and

other fixed costs) and an international sale price of US\$3,600 per kilogramme, it is estimated that the annual gross profit would be in the region of US\$580,000. Under the agreement with M/s FDC Ltd., RRL-Jorhat is paid royalties of 1 per cent of the sale price, amounting to more than US\$8,000 each year.

PARTNERSHIPS

The technology for producing pure β -arteether was developed as an in-house programme and scaled up to deal with a charge of 5 grammes of artemisinin per batch. However, to further scale up the process to the pilot-scale level while optimizing the different parameters required additional financial support. At this stage, the technique was reported in the RRL brochure and annual reports. Responses were received from several private-sector Indian pharmaceutical companies. Eventually, an agreement was reached with one of these, M/s FDC Ltd., Mumbai.

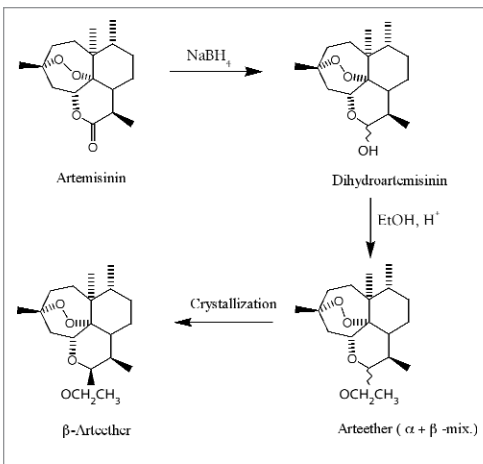


Figure 1 | Chemical synthesis of β -arteether from artemisinin.

REPLICABILITY

Malaria is a serious disease in many parts of Africa, Asia, Latin America and Oceania, affecting 5 per cent of the population at any time. Some 2,100 million people are at risk of the disease and 270 million people are infected each year. In a 1990 report, WHO conservatively estimated the worldwide mortality rate to be one million people per annum, but a recent review indicates that malaria may cause several million deaths each year. The resistance of the malaria parasite to chloroquine has led WHO to predict that, without new antimalarial drug intervention, the number of cases of malaria will double by the year 2010.

In view of this, the production of chirally pure β -arteether is relevant to all regions where malaria is endemic. This plant-to-pharmaceutical-product technology can be replicated in any other malaria-affected country.

POLICY IMPLICATIONS

There is no direct change in legislation due to this innovation, but as India increasingly focuses on export-led growth and competition in the global marketplace, it is clear that the nation's research and development strategy must be able to produce products and processes that meet international standards in terms of quality, cost and efficacy. Under these circumstances, this innovative experience is likely to have an impact beyond India's national boundaries.

LESSONS LEARNED

Analytical-grade solvents and chemicals are costly and are not used in commercial plants. Any process parameter optimized using analytical-grade chemicals will therefore be difficult to translate into commercial-scale production. In this case study, therefore, only commercial-grade bulk packs were used from the very beginning.

The process developed for producing chirally pure β -arteether is based on artemisinin procured from commercial sources and the steps involved in the process have been optimized using artemisinin of a pre-defined purity. As the purity profile of artemisinin extracted from plants is likely to vary from batch to batch or from source to source, the process may also need to be modified slightly in order to get a final product of suitable quality.

WHO recommends a 40:60 mixture of α and β isomers of arteether for clinical use although it is known that only the β isomer is effective as a drug and not the α isomer. Despite being able to produce purified β -arteether, the Drug Controller (India) refused to grant a permit for the preparation as there were no available data on the toxicity of the isolated isomer. This delayed the commercialization of the product. Acute and sub-acute toxicity data on the β isomer were generated with the help of the commercial sponsor, M/s FDC Ltd. The absence of an association with a sponsor during the development stage of the product would have led to further delays.

IMPACT

It is abundantly clear from WHO figures that a new-generation antimalarial drug available at a lower price will save millions of lives around the world. India, being a high-risk region for malaria, urgently requires such a drug. The commercial availability of β -arteether, therefore, will have a qualitative impact on the health of Indian society.

Furthermore, as arteether has a different mode of action from other antimalarial drugs and there are no reports of malaria parasites acquiring resistance to it, it is expected to remain on the market for a long time.

Although arteether is currently available on the market, it is available as a 40:60 mixture of α and β isomers. To date, there are no reports of any adverse effects being caused by the α isomer. However, there is a growing awareness that some racemic drugs (i.e., those containing mixtures of both isomers) can have adverse side effects and this may hamper the acceptance of the presently used α and β mixture of arteether. As an effective alternative, chirally pure β -arteether is likely to replace the racemic arteether in the near future.

FUTURE PLANS

The technology for β -arteether developed by RRL-Jorhat has been sold to M/s FDC Ltd., Mumbai, on an exclusive basis for marketing in India and abroad. Although all the steps involved in the process have been optimized and the overall process is highly profitable, there is still scope for further improving the process. Among the steps where refinements could be made are:

- reducing the quantity of solvent required;
- reducing the consumption of raw materials;
- replacing the methanol solvent with water; and
- replacing the batch crystallization process of separating the α and β isomers of arteether with a continuous crystallization technique.

PUBLICATIONS

Bez, G., Kalita, B., Sarma, P., Barua, N.C. and Dutta, D.K. (2003). Recent advances in the chemistry of 1,2,4-trioxane type artemisinin analogues (a review). *Current Organic Chemistry*, 7:1231-1255.

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