Alpha/beta arteether, a new antimalarial

Plasmodium falciparum is the predominant parasite species in the northeastern states comprising over 70% of the malarial infections transmitted by *Anopheles minimus*, the major vector in the region[1]. Malaria outbreaks are of frequent occurrence and morbidity and mortality are on the rise largely due to *P. falciparum* (the dreaded form of malaria). The problem is further compounded due to the widespread decreased sensitivity to commonly-used antimalarials[2]. Thus the development of alternate drugs is timely and appropriate. In this context, the derivative of Chinese herb qinghaosu (*Artemisia annua*) including artemisinin, artesunate and artemether have been reported to be effective schizontocidal drugs[3].

To further the research in this direction, Central Drug Research Institute (CDRI), Lucknow in association with Central Institute of Medicinal and Aromatic Plants (CIMAP) has taken up the task of development of ethyl ether derivative of artemisinin, namely alpha/beta arteether, for convenient parenteral treatment of severe and complicated form of *falciparum* malaria. This is a racemic mixture and has added advantage over its analogues due to its increased solubility in oil medium and is more economical for large-scale production. This preparation was found to be very effective against *P. knowlesi* and *P. cynomolgi* in rhesus monkeys and phase I and II clinical trials have been successfully completed for treatment of human *P. falciparum* malaria[4–6]. In phase III multicentric trials, alpha/beta arteether was put to field evaluation for its curative efficacy against complicated and uncomplicated *P. falciparum* cases in different malaria endemic pockets in India including Assam in the northeastern region. Included in this communication are the effects of this drug on asexual and sexual stages of *P. falciparum*.

In northeastern India, a study was conducted in Assam in collaboration with Central Reserve Police Force Base Hospital, Guwahati (District Kamrup) and Paneery Tea Estate Central Hospital (District Darrang) as per WHO guidelines for extended follow up for drug sensitivity on human subjects. An informed consent was obtained from enrolled patients. The drug was administered deep intramuscularly in the gluteal region at a dose of 150 mg once daily for 3 consecutive days (D0, D1, D2). A total of 41 febrile patients (27 males + 14 females) having moderate to acute *P. falciparum* parasitaemia ranging from 8480 to 3,20,000 per cubic mm of blood were selected for the follow-up study. Of these, 26 were categorized as uncomplicated and 14 complicated as per protocol developed for this purpose based on the clinical presentation of each patient. These comprised various ethnic tribes of Assam namely Karbi, Boro, Rabha, Lalung and tea tribes; a fair representative of cross-section of society in the age group of 14 to 50 years. Peripheral blood smears were taken every day from day 0 to 7, day 14, day 21 and day 28, for parasitological examination/parasite count of asexual and sexual stages.

It was observed that in 34 patients (83% of the cases) asexual parasitaemia were eliminated within 24 h; in 6 patients (15% of the cases), it took 48 h. Cure rate was 100%. Along with asexual parasitaemia, sexual stages were also observed in three patients, and its density varied from 8 to 6400 per cubic mm of blood. Gradual decline in gametocyte count was observed in two patients and it disappeared by day 14. In one patient, however, the level of gametocyte count remained the same throughout the study period. The gametocytocidal effect of alpha/beta arteether could thus not be clearly ascertained. Recrudescence was not recorded within 28 days of follow up study. Fever clearance time ranged between 36 and 96 h. Based on other clinical parameters/adverse reaction monitoring thereof (data to be presented elsewhere), alpha/beta arteether was concluded to be a safe, well tolerated, highly effective schizontocidal, fast-acting convenient drug, and it holds good promise for management of acute *P. falciparum* cases. The intra-muscular preparation has an added advantage over oral therapy for ensuring compliance in ethnic tribes for effective treatment. Similar observations were recorded in tea tribes of Upper Assam[7].

The phase III multicentric clinical trials have now been concluded in different regions of India with varying malaria endemicity[8]. Based on the field evaluation in these areas on 478 (267 uncomplicated + 211 complicated) patients, alpha/beta arteether has now been approved by Drugs Controller of India for restricted circulation as an alternative treatment for multi-drug resistant *P. falciparum*/cerebral malaria cases. Further, the 22 clinical trials from 6 countries have been reviewed to compare the results with the Indian experience[9]. The mean parasite clearance time compared well with results from other countries while the recrudescence rate observed in the Indian studies was the lowest.

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Targeted transgene integration: The way ahead to stable transformation

Stable integration and expression of introduced gene is essential to realize transgene advantage in the genetically-engineered crops. However, it has been clear for sometime that the introduced genes are not expressed uniformly in independent transgenic plants and that transgenic expression can change in successive generations. In addition, introduced genes can suppress the expression of related endogenous genes and/or transgenes already present in the genome[1]. The ability to recognize self, from non-self, a characteristic of the immune system, and the existence of self-DNA protection systems might modify the foreign DNA to make it nonfunctional or eliminate it altogether.

Although several factors contribute to inactivation and elimination of intrusive nucleic acids, methylation of intruder DNA and homology-dependent ectopic pairing are probably the major factors that contribute to transgene inactivation and gene silencing. The integration intermediates may be the prime targets for DNA methyltransferases that enforce repression of transgenes. Also, evidence is rapidly accumulating that silencing of single copy foreign genes, multicopy transgenes integrated either at the same locus or at unlinked loci frequently cause silencing of themselves and of homologous host sequences. The frequency of silencing encountered in multicopy transformants has led to the speculation that enhanced DNA : DNA pairing of the repetitive elements in such complex inserts might act as a signal for detection, resulting in highly efficient silencing. Therefore, suitable transformation constructs need to be designed to avoid host surveillance processes and facilitate predictable integration.

The preferred sites of transgene integration

The intrinsic ability of viral sequences or mobile genetic elements to excise and reinsert several times in search of a compatible genomic environment to attain stable integration is probably a mechanism for escaping the self-protection system of the host. The investigations into the sequence complexity of a genomic organization suggest that genomes are made up of 'isochores', i.e. very long stretches of DNA with high compositional homogeneity[2]. Carels *et al.*[3] found that almost all genes in maize are present in isochores covering an extremely low GC-range (1–2%) that represent only 10–20% of the genome. From this discovery, they developed the concept of 'gene space', in which genomic regions are represented by a single family of isochores[3], and noted that the gene space in maize corresponded to the only genome compartment in which certain mobile sequences can be transposed. The implication is that exogenous DNA arriving within the GC-rich gene space is likely to be actively transcribed, whereas DNA inserted into other regions is unlikely to be transcribed. Axiomatically, the GC-rich transgenes inserted into transcriptionally-active DNA remain functional, whereas AT-rich sequences in transgenes may mark them for inspection as invasive DNA. A precedent for this generalization has been observed in mammals: mobile sequences that have undergone numerous amplification and translocation events during the evolution of mammals, and several integrated viral sequences, were predominantly found in isochores of matching GC-composition[2]. Thus, it can be inferred that a matching genomic environment is essential for stable integration and expression of inserted sequences, and that excision and reinsertions may continue until the candidate transgene finds a compatible gene space in the host genome, else it would be eliminated/inactivated through the genome scanning mechanism[4]. The observations recorded from isochore studies[2,3] suggest that genomic systems exist that facilitate identification and inactivation (or elimination) of the foreign sequences that are compositionally different or incompatible from the genomic sites into which the insertions take place.

Iglesias *et al.*[5] have made an important discovery in relation to the stable integration and expression of transgene from the physical mapping of introduced DNA with respect to the spatial sites and sequence composition of chromosomal segments. Using fluorescence *in situ* hybridization to probe the physical location of transgene insertion, they demonstrated in tobacco that the stably-expressed inserts were present in the vicinity of telomeres, whereas the unstably-expressed inserts occupied intercalary and paracentromeric locations. Also, the stably-expressed loci comprised relatively simple T-DNA arrangements that were flanked on at least one side by plant DNA containing AT-rich regions that bind to nuclear matrices *in vitro*. It may be important to