

inserted precisely into the genome of *Anopheles* cell lines. The transformed insect was identified from other mosquitoes, by placing the green fluorescent protein gene marker. When viewed under ultraviolet light, the green light became visible to the eye. Monitoring by fluorescence of the green light was a good indicator of successful transformation.

Well, all was not so straightforward. For a successful injection of an exogenous gene into the mosquito egg, to create the transgenic malaria mosquito, hardening of the membrane around the mosquito egg poses a problem. To circumvent this, the chemical *p*-nitrophenyl *p'*-guanidinobenzoate, an inhibitor to the hardening process was employed in the solution used to lay mosquito eggs. By this simple method, injections were possible into

the eggs as they remained soft and transparent.

On average, 29% of injected embryos survived and around 50% of the hatched larvae were detectable by fluorescence. Survival to the adult stage was on an average 10% showing successful transformation. The transformation frequency obtained was higher than in previous transformation experiments reported in *Drosophila melanogaster* and *Ceratitis capitata* using *Minos* marked with the white gene marker.

Precision of transposition was verified by determining the sequences of the junctions between the inserted element and the *A. stephensi* genome. Genomic DNA libraries from three families were screened with a probe. All positive clones were sequenced and the presence of precise *Minos*-mediated integrations in these three families were confirmed.

In situ hybridizations performed, detected a single insertion site per chromosome complement, and this site was distinct in each family.

The work of Catteruccia *et al.* demonstrates that on the future malaria control map, substituting other genes could make the malaria carrying *Anopheles* mosquito produce antibodies to the malaria parasite or a resistance to it. Thus creating a safe mosquito unable to transmit malaria and making a major breakthrough in molecular entomology.

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RESEARCH NEWS

A new, broad-spectrum anti-tubercular drug on the horizon

Pawan Sharma

Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), has emerged as the single most devastating, global pathogen. With nearly eight million new cases of active disease and three million deaths on its account every year, TB has displaced malaria to claim the dubious distinction of becoming the topmost killer infection of the world today¹. In India alone, TB accounts for nearly half a million deaths from nearly two million new cases each year, and as such represents a large proportion of the global TB burden. Furthermore, it is estimated that one third of the entire human population is infected with Mtb. This large population with latent infection serves as a reservoir for continuous emergence of fresh cases with active disease as epidemiological studies have indicated that nearly 10% of these would develop active disease sometime in their lifetime². Unfortunately, the disease is expected to

continue to spread without respite unless novel diagnostic, treatment and intervention measures become available. The only anti-TB vaccine available today, viz. the Bacilli Calmette-Guerin (BCG) has had little impact on the overall TB prevalence worldwide³. The current anti-tubercular drugs are toxic and limited in their activity and the treatment regimens are extremely long and difficult to sustain. The pandemic of HIV infection which dramatically increases susceptibility to develop active TB and the emergence of drug resistant strains of Mtb in several parts of the world have further exacerbated the situation⁴. There is, therefore, an urgent need for developing new vaccines and drugs to stem the spread of TB.

Until about fifty years ago, no drug was available for treatment of TB. Today, Mtb strains resistant to several drugs have appeared all over the world. The fact that no new drug with novel

mechanisms of action against the tubercle bacillus has appeared in the last three decades, underlines the tough challenge posed by this pathogen. Against this background, it is heartening to come across a recent report by Stover and colleagues⁵ about development of a new, small-molecule drug candidate. Its unique anti-tubercular properties make it far superior to the current anti-TB drugs. This compound belongs to the family of bicyclic dinitroimidazoles originally developed as radio-sensitizers in cancer chemotherapy⁶. These were found to possess anti-tubercular activity as well⁷, but because of their mutagenicity, were not investigated subsequently for further development. Stover *et al.*⁵, however, synthesized a series of more than 300 3-substituted nitroimidazopyran (NAP) compounds and found that more than 100 of these possessed substantial, specific activity against the tubercle bacil-

lus. Significantly, none of the active compounds had any indication of mutagenicity. Furthermore, the activity of these compounds was directed specifically against the pathogenic mycobacteria belonging to the Mtb complex such as *M. tuberculosis*, *M. microti*, *M. fortuitum*, etc. with virtually no activity against the non-tuberculous mycobacteria (NTM) like *M. avium*, *M. smegmatis*, etc. the environmental mycobacteria which are generally non-pathogenic to humans.

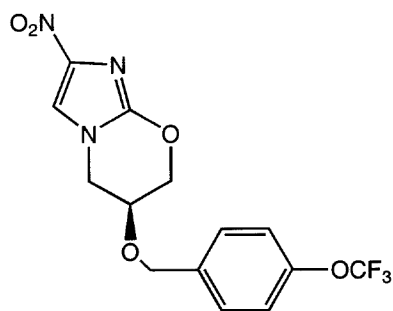
Out of the several NAP compounds that showed high antitubercular activity, Stover *et al.*⁵ chose the one named PA-824 (Figure 1) for further studies because of its promising therapeutic activity in the short-term murine model as well as in the aerosol challenge guinea pig model of the disease. On oral administration, this compound exerted bactericidal activity against virulent Mtb comparable to that of the frontline anti-TB drug, isoniazid (INH), at doses which were safe in mice and guinea pigs. In the *in vitro* assays, the minimal inhibitory concentration (MIC) values

of PA-824 were found to be in the sub-micromolar range, varying from 0.015 to 0.25 $\mu\text{g ml}^{-1}$ against a dozen of pan-sensitive or mono(rifampin)-resistant clinical isolates of Mtb. More significantly, it also exhibited similar effect on a number of genetically distinct, multi-drug resistant (MDR) strains of Mtb, and the pattern of bactericidal activity indicated that there was no cross-resistance with several antitubercular drugs in current usage such as INH, rifampin, streptomycin, ethambutol, ethionamide, kanamycin, pyrazinamide, etc. Incidentally, this observation also provides support for the continued usefulness of these drugs in judiciously designed treatment strategies.

But the most important advance reported in this paper relates to the significantly high neutralizing activity of PA-824 against the non-replicating Mtb in the microaerophilic cultures. The latter provide an *in vitro* model system for the latent form of Mtb. It is well known that even those Mtb strains which are susceptible to available anti-TB drugs can enter a non-replicating, dormant phase and hide away in low-oxygen regions of host tissues; and no anti-TB drug in current use has any significant effect on these forms. Since people with latent TB (and they constitute one-third of the entire human population!) are largely responsible for continued high incidence of the disease, the new compound, PA-824, holds strong promise of cutting down the incidence to a very significant extent.

The compound PA-824 was found to act in part by inhibiting a critical step in the oxidation of hydroxymycolate to ketomycolate, a critical component of the mycobacterial cell wall. It is not clear as yet if this effect was mediated by inhibition of an enzyme, or, by de-

pletion of a co-factor essential for this step in the biosynthesis of the cell wall. There is some indication, though, that PA-824 exerted its lethal effect through concurrent inhibition of protein synthesis as well. In conclusion, Stover *et al.*⁵ point out that as a small, orally active molecule amenable to large-scale synthesis and with demonstrable activity against MDR strains as well as non-replicating static forms of Mtb, PA-824 offers a powerful new tool to be developed for use in the antitubercular therapy.



PA-824

Figure 1. Structure of PA-824.

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