

Nobel shot in the arm for neglected infectious disease research

In the era of genomics, proteomics, metabolomics, synthetic biology and systems biology, the award of Nobel Prizes (2015) for neglected infectious disease research is a bit surprising, even out of tune. It is not only a reminder of the major public health issues facing developing countries in particular, but also a signal to researchers in these areas in developing countries that they can hope for the highest academic recognition in addition to a greater recognition by the society at large, if they can contribute to alleviation of human suffering. One does not have to jump into the bandwagon to do fashionable research, but significant contributions based on traditional paths can also lead to very fruitful outcome. Modern tools can help a great deal but ideas and commitment are important. The diseases covered under the award alone account for close to half-a-billion infections and 3–4 million deaths in a year.

Artemisinins

Youyou Tu, from China, was awarded one half of the prize money for ‘her discoveries concerning a novel therapy against malaria’. She is credited with the discovery of artemisinin, a drug that has contributed to a significant decrease in mortality due to malaria. The present incidence of malaria is around 250 million cases and close to 600,000 deaths in a year, mostly accounted for by children in Africa. Although a large number, it

represents a 50% reduction due to several public health measures such as the use of pyrethroid-coated bed nets, indoor spraying, etc. along with ART (artemisinin derivatives)-based therapy to cure the disease. Youyou Tu was born in 1930 in China and is a Chinese citizen. She graduated from the Pharmacy Department at Beijing Medical University in 1955. Her professional career has been at the China Academy of Traditional Chinese Medicine and has been Chief Professor, since 2000. She was not a member of the prestigious Science or Engineering academies in China.

Malaria is due to five *Plasmodium* species with *P. falciparum* and *P. vivax* contributing, perhaps to 95% of the patients. *P. falciparum* accounts for 90% of the mortality, although more virulent forms of *P. vivax* are being reported in recent times in addition to causing morbidity and loss of man hours due to prolonged affliction. *P. falciparum* has become resistant to chloroquine, antifolates and even to quinine in some cases and ART remain the only hope. This is the first Nobel prize to a Chinese scientist, who did all the work in China. Interestingly, artemisinin was discovered by the order of Mao Zedong under the secret code project 523 to help North Vietnamese soldiers dying in the war against the US due to chloroquine-resistant malaria infection. The period was the 1960s, when the scientists were shunted out to villages during the period of Cultural Revolution. But, this project because of diktat from Mao himself survived and

some 500 scientists in many institutes were involved. There was a controversy as to who should share the credit for discovering artemisinin, when Tu received the Lasker award (considered as US Nobel) in 2011. There is a consensus that she deserves the award because she led the team at the Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences (CACAMS). The project was started in 1967. In her own words¹ – ‘The only reference relevant to use of qinghao (the Chinese name of *Artemisia annua* L.) for alleviating malaria symptoms appeared in Ge Hong’s *A Handbook of Prescriptions for Emergencies* (284–346 CE): “A handful of qinghao immersed with 2 liters of water, wring out the juice and drink it all.” This sentence gave me the idea that the heating involved in the conventional extraction step we had used might have destroyed the active components, and that extraction at a lower temperature might be necessary to preserve antimalarial activity. Indeed, we obtained much better activity after switching to a lower temperature procedure.’ She screened 200 recipes with Chinese traditional herbs and 380 extracts from the herbs were tested in *P. berghei*-infected mice and *P. cynomolgi* infected monkeys, before success was achieved with *Artemisia annua* (sweet worm-wood). She devised the appropriate extraction procedure, eliminated the acidic portion and obtained a neutral extract that had 100% killing activity against *P. berghei* in mice. The group ensured that the extract

Artemisia annua plant



Sesquiterpene lactones with unusual peroxide bridge

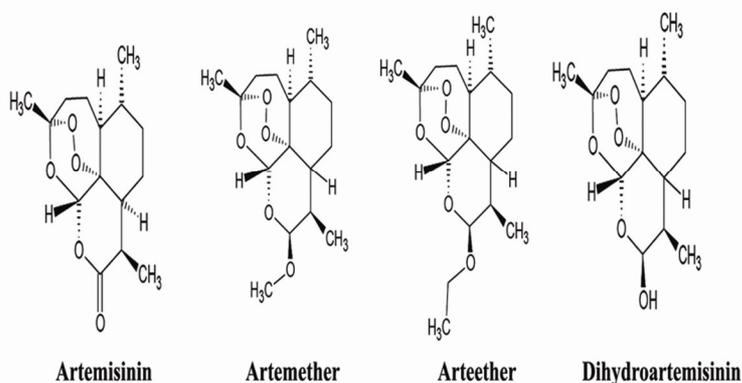


Figure 1. Structure of some of the artemisinin derivatives. Courtesy: Photographed by Kristian Peters.

was safe by experimenting on themselves before demonstrating that it is very effective in clearing the parasite in *P. falciparum* and *P. vivax*-infected patients. A colourless crystalline compound with a molecular formula $C_{15}H_{22}O_5$ and melting point of 156–157°C was characterized in 1972 as the active component and named quinghosu (basic element) or artemisinin. She also worked on a better tablet formulation. Other colleagues/groups were involved in determining the stereo-chemistry of this sesqui-terpene lactone and identifying a better variety of *A. annua* from Sichuan province with higher artemisinin content. Her group also identified dihydroartemisinin to be more stable than artemisinin. Various derivatives of artemisinin are used according to requirement (Figure 1).

The first publication was in 1977 in a Chinese journal². There was embargo to publish work on artemisinin outside China. The first presentation was made to the Scientific Working Group on the Chemotherapy of Malaria (CHEMAL) of the WHO in 1981 in Beijing. Some years later, I also visited some of the artemisinin-extraction plants in Beijing as a member of the CHEMAL group. At some point of time, the US tried to promote mefloquine as an alternative to artemisinin, but it cannot match and is more toxic. WHO did not endorse ART till 2000 and it was not widely available till 2006.

Mechanism of action and resistance

It is a matter of great concern that in the last few years evidence is building up for the detection and spread of resistance to ART, especially in the Cambodia, Thailand and Myanmar border areas. It will be disastrous if it spreads to Africa, which is the epicentre of *falciparum* malaria. India is quite vulnerable with its lack of proper estimates of malaria deaths, with reports ranging from 1000 to 40,000 per year! Someone remarked that the 1000 deaths estimate should be from a single district! The molecular mechanism of action of ART is not fully understood. It seems to act at multiple sites. It inhibits haemoglobin uptake and digestion by the parasite. The host red cell haemoglobin provides amino acids for parasite sustenance. The haeme released from haemoglobin is toxic to the parasite and so is inactivated by the parasite

through the formation of haemozoin, a biocrystallized form of haeme adducts. ART also interact with haeme-iron derived from haemozoin pigment, resulting in the scission of the endoperoxide bridge. This could lead to free radical generation and the net result seems to be inactivation of several proteins, including PfATP6, flavoenzyme and mitochondrial electron transport chain of the parasite. There is also a frantic effort to understand the molecular basis for the emerging resistance development to ART. These studies have led to the identification of mutations in the gene encoding 'propeller' domain of Kelch protein, correlating with resistance developed in the laboratory strains and field isolates. Additional mutations are not ruled out. Polymorphisms seen in this gene seem to be different between Southeast Asian population and sub-Saharan Africa. This knowledge would help track the spread of resistance to ART. ART monotherapy has to be for 7 days and failure to adhere to the regimen and use of substandard drugs are the main reasons for resistance development. WHO has insisted on combination therapy since 2005 on the basis that while ART has a very short half-life, the combining partner will have a longer half-life to account for the residual parasites and this could delay evolution of resistance. The Swiss company Novartis bought the new Chinese patent for artemether-lumefantrine combination and took western patents before commercialization. Combinations such as artemether-lumefantrine (Coartem), dihydroartemisinin-piperaquine (Euartesim), etc. are available at subsidized costs in Africa due to availability of funds through Global Fund to Fight AIDS, tuberculosis and malaria in 2002 and the Bush administration's introduction of the President's Malaria Initiative in 2005. It is a matter of concern that resistance could develop to these combinations as well. We have developed arteether-curcumin combination, which we believe is superior to others, as curcumin can prevent development of resistance, decrease the toxicity of the primary drug and is very effective in Experimental Cerebral Malaria in mice³. It is awaiting DCGI approval to carry out efficacy trial. Nityanand at Central Drug Research Institute (CDRI), Lucknow developed the alpha, beta-arteether version that is cheaper than beta-arteether. The Central Institute for Medicinal and Aromatic

Plants (CIMAP), Lucknow has played an important role in the cultivation of *Artemisia annua* in India with higher production levels.

While China is legitimately proud of its achievement, a debate has broken out in China on the efficacy of Chinese medicine, reminiscent of the debate on Ayurveda in India. While one group feels that the Nobel Prize is a vindication of the efficacy of Chinese medicine, another group feels that the Chinese medicine is usually a combination of 10–20 herbs or minerals adjusted on a constant basis based on patient response without any knowledge of the mechanism of action and it would be dangerous to follow this path. He Zuoxiu, a member of the Chinese Academy of Sciences, says 'I think for the future development of Chinese medicine, people should abandon its medical theory and focus more on researching the value of herbs with a modern scientific approach'. Well, this statement can become a matter of debate in India as well with respect to Ayurveda and other forms of traditional medicine!

Avermectins

William C. Campbell and Satoshi Ōmura received the other half of the Nobel Prize for their discoveries concerning a novel therapy against infections caused by roundworm parasites. They discovered Avermectin (Figure 2), the derivatives of which have radically lowered the incidence of River Blindness and Lymphatic Filariasis and also used extensively to treat parasitic diseases of cattle and other animals. William C. Campbell was born in 1930 in Ireland and received his BA from Trinity College, University of Dublin, Ireland in 1952. He got his PhD from University of Wisconsin, USA in 1957. From 1957 to 1990 he was with the Merck Institute for Therapeutic Research, and from 1984 to 1990 as Senior Scientist and Director for Assay Research and Development. Currently he is a Research Fellow Emeritus at Drew University, Madison, New Jersey, USA. Satoshi Ōmura was born in 1935 in the Yamanashi Prefecture, Japan and is a Japanese Citizen. He received a Ph D in Pharmaceutical Sciences in 1968 from University of Tokyo, Japan and a Ph D in Chemistry in 1970 from Tokyo University of Science. He was a researcher at the Kitasato Institute, Japan from 1965

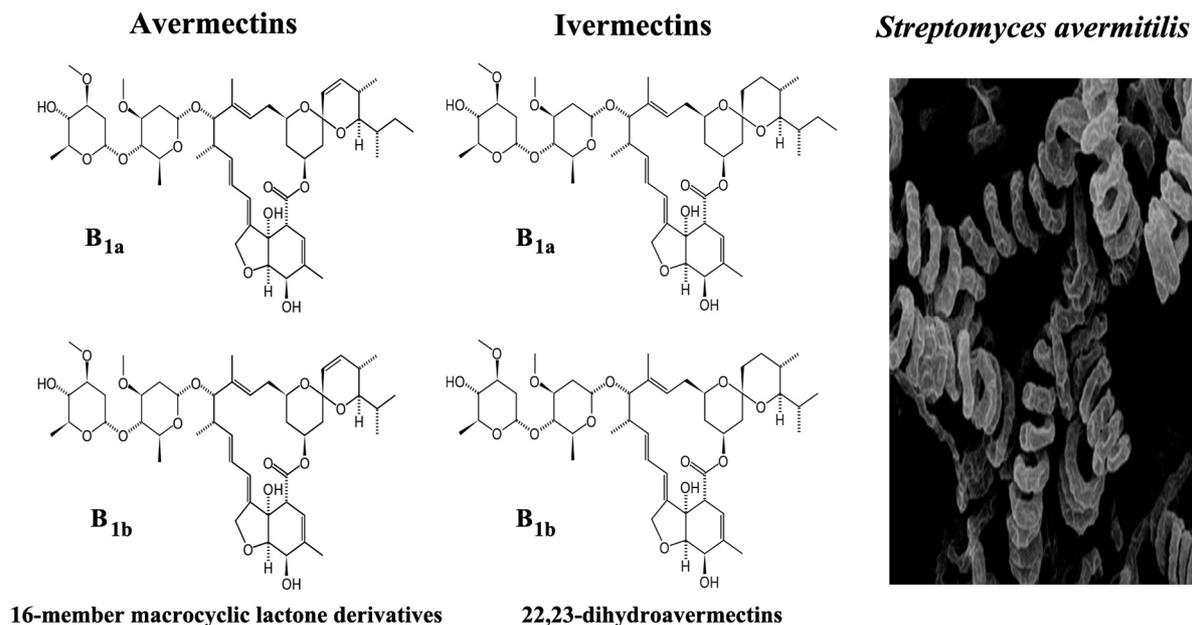


Figure 2. Structure of avermectins and ivermectins. Courtesy: Ikeda *et al.*⁷.

to 1971 and Professor at Kitasato University, Japan from 1975 to 2007. From 2007, Satoshi Omura has been Professor Emeritus at Kitasato University.

The discovery of Avermectin represents an excellent example of a successful public-private-partnership in today's jargon. In the early 1970s, Omura, then head of the antibiotics research group at Kitasato University, Japan initiated a collaboration with Merck, Sharpe and Dohme (MSD) in the US. Microorganisms were isolated from Japanese soils and evaluated *in vitro* for bioactivity. The promising samples were then analysed *in vivo* at MSD. Among thousands of *Streptomyces* cultures, the most promising 50 were sent by Omura to MSD, where William Campbell isolated a compound from one of the cultures, which was remarkably efficient against parasites in domestic and farm animals. The compound was named Avermectin and it was subsequently converted to the more active derivative ivermectin (Figure 2)^{4,5}. The joke is that Omura was fond of the golf course and *Streptomyces avermitilis* is from the soil nearby! Ivermectin was introduced as a commercial product in 1981 for animal health. It is active against a wide range of parasites, including gastrointestinal roundworms, lungworms, mites, lice and hornflies. It is also highly effective against one of the most important cattle parasites in the tropics and subtropics, namely ticks (ixodid tick

Rhipicephalus (Boophilus) microplus). Presently, ivermectin is being used to treat billions of livestock and pets around the world.

I am providing a gist of the story described by Crump and Omura regarding human applications⁶. Ivermectin has now become a wonder drug as an essential component in the campaign to eliminate, onchocerciasis and lymphatic filariasis, two of the most devastating and disfiguring diseases of the human. Onchocerciasis (River Blindness) is due to *Onchocerca volvulus* worms transmitted through the bites of blackflies (*Simulium* genus). The vector breeds in highly oxygenated, fast-flowing rivers. Immature forms of the parasite mature into adult forms in the subcutaneous tissue of the human. The microfilariae released by the female worms in several hundreds move through the body and cause a variety of disfiguring skin lesions. They also invade the eye and cause vision impairment and blindness. They cause blindness in 1–2 million people. The burden was very severe in the sub-Saharan belt. The saga of the rescue of 30 million people in 11 countries across 1.2 million sq. km against the disabling and disfiguring disease launched by four United Nations agencies, including the World Bank through the Onchocerciasis Control Programme (OCP) in West Africa in 1974 is laudable. The entire effort was spearheaded by the UN-based Special Pro-

gramme for Research and Training in Tropical Diseases (TDR), established in 1975.

Onchocerca volvulus would only develop fully in the human and a few primates and the latter could not be used for screening due to ethical reasons. It was found that cattle were infected by *O. ochengi*, a close relative, also by the same vector. TDR provided technical and financial support to establish primary and secondary screens for *Onchocerca filaricides* in five academic and private research institutions with technical capacities and facilities: the University of Georgia (USA), University of Giessen (Germany), the Wellcome Foundation (UK), the London School of Hygiene and Tropical Medicine (UK) and the University of Tokyo (Japan). In addition, a tertiary screen in cattle was established at James Cook University of North Queensland, Australia. Some 10,000 compounds supplied by the pharmaceutical companies were screened. At that time only two drugs were available to treat River Blindness, Diethylcarbamazine (DEC) and Suramin. Both were unsatisfactory due to hypersensitivity and toxicity.

It was not easy for ivermectin to make the grade. Initially, TDR was not very enthusiastic about ivermectin, as it was looking for a macrofilaricide and the molecule did not seem to have much effect on adult worms. However, Merck was moving ahead with its own research to phase

I trials (safety) in Senegal, where it was found that the drug at 30 µg/kg could clear skin microfilariae and was safe at least up to 200 µg/kg. The effect lasted for at least 6 months. In 1982, TDR and OCP realized the enormous potential of ivermectin and community-based clinical trials could be conducted with the participation of all the partners, although collaboration was started 'in a complex environment of mutual wariness, suspicion and shared hope that ivermectin would indeed prove to be an effective treatment for Onchocerciasis'. In addition, Merck looked at ivermectin as a commercial product to be priced at 6 USD a dose, whereas TDR looked at it as a community-based tool to interrupt parasite transmission. In July 1985, the company decided to make the drug available to 'governments and patients at no cost to them for the treatment of Onchocerciasis'. Each year, Merck gives away some 270 million treatments of the drug, according to the Mectizan Donation Programme, in Decatur, Georgia. DEC is also effective in clearing the parasite, but causes ocular damage due to inflammatory response. Avermectin has become a tailor-made molecule to treat onchocerciasis.

Lymphatic Filariasis

The name Elephantiasis derived from swollen legs and genital organs is a debilitating and disfiguring disease affecting 120 million people, of whom 40 million are seriously affected. The disease is transmitted through mosquito bites delivering filarial worms, *Wuchereria bancrofti*, *Brugia malayi* or *B. timori*. The adult worms develop in the lymphatic vessels and the female gives rise

to thousands of microfilariae, causing lymphoedema. Once again Merck and TDR undertook broad clinical trials in several countries including India with ivermectin, DEC and combination. Both the drugs were effective in a single dose and the combination was effective even at a lower dose. The drug itself could be registered only in 1998. Subsequently GSK introduced albendazole, effective in killing immature and adult worms. With the availability of three effective drugs, namely ivermectin, DEC and albendazole as donations, WHO is on a Global Programme to Eliminate Lymphatic Filariasis (GPELF).

Mechanism of action and resistance

Ivermectin has been shown to disrupt GABA (Gama-aminobutyric acid) receptor in invertebrates and mammals with glutamate-gated Cl⁻ channel as the target. These receptors, found exclusively in invertebrates, belong to the pentameric Cys-loop receptor family of ligand-gated ion channels (LGICs). GABA is recognized as the primary inhibitory neurotransmitter in the somatic neuromuscular system of nematodes. Avermectin paralyzes body-wall and pharyngeal muscle in nematodes, but does not affect mammals, since it cannot cross the blood-brain barrier. Interestingly, it does not have a significant effect on microfilariae in culture. The body of evidence indicates that the drug may be acting by suppressing the release of proteins from the nematode that help in evading the protective Th2 response of the host defense mechanism. Ivermectin resistance has been known in animal parasites, especially cattle. It is still not an issue in the human, but there

is a concern that resistance in parasites may be selected due to under-utilized public health strategy. Therefore, there are efforts to make derivatives and implement combination therapies to prevent development of resistance. The complete genome sequence of *Streptomyces avermitilis* is available and that would help tracking resistance markers.

Hope for mankind

The process of discovery of artemisinin and avermectin should be a great inspiration to researchers working with neglected infectious diseases with many of them killing millions in developing countries. In the midst of global concerns regarding local wars and terrorism, it is heart-warming that scientific discoveries can make a difference to the alleviation of human suffering and individuals and organizations, be it public or private, will rise to the occasion to save mankind.

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