Novel inhibitor to combat kala-azar identified

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Combining structure-based drug designing methodology with in vitro studies, scientists have been able to identify a FDA-approved molecule that shows enhanced anti-kala-azar activity.

Three active inhibitor molecules were selected from the PubChem database and one of them showed the highest stability in binding to the active sites of the target enzyme (UDP-galactopyranose mutase or UGM) which helps in the formation of glycoprotein, beta-Galf. After binding to the UGM, the molecule inhibits the enzyme activity thereby reducing the virulence, parasite survival and transmission of disease. The results were published in the *Journal of Cellular Biochemistry.*

Limited treatment

Treatment for kala-azar (disease caused by *Leishmania* infection) is limited due to high toxicity to human cells, low efficacy of the drug, high cost and drug resistance making the development of novel anti-kala-azar drugs a priority.

India has around 3,000 people afflicted with kala-azar, accounting for 50% of the global burden. It is endemic in West Bengal, Bihar, Jharkhand and eastern Uttar Pradesh.

Beta-Galf is a major cell surface component of *Leishmania* parasite and is responsible for the virulence of the pathogens and plays an essential role in parasite survival and transmission of disease. Beta-Galf is also found in *Mycobacterium tuberculosis* that causes TB and *Trypanosoma cruzi* parasite that causes sleeping sickness but is absent in humans. Like beta-Galf, the UGM enzyme is also absent in humans but is critical for the biosynthesis of beta-Galf thereby making the UGM enzyme an attractive drug target. Deletion of the gene encoding for the enzyme in *L. major* resulted in a decrease in virulence.

Since the protein structure of *Leishmania* UGM is not known, Dr. Yusuf Akhter and other scientists used the protein structure of *T. cruzi* UGM as a template and the protein sequence of *Leishmania* was modelled on the template. “There is 60% sequence identity between *Trypanosoma* UGM and *Leishmania* UGM,” says Dr. Akhter from the School of Life Sciences, Central University of Himachal Pradesh, Kangra, Himachal Pradesh and one of the corresponding authors of the paper.

In vitro studies

One of the three chosen inhibitors was evaluated *in vitro* for anti- *Leishmania* activity and found to significantly inhibit the growth of *Leishmania donovani* (which causes damage to visceral organs such as liver and spleen). Different doses of the compound were tested and the minimum inhibitory concentration or IC50 value (the lowest concentration of the compound required to inhibit the visible growth of a pathogen) was found to be 50 microgram per litre. The IC50 value of the approved drug miltefosine hydrate is only 25 microgram per litre.

But the approved drug miltefosine hydrate showed 100% toxicity to human cells when 50 microgram per litre was used whereas the toxicity of the screened molecule was only 50% at the same concentration. The toxicity of miltefosine hydrate was as high as 89% even when 25 microgram per litre (which is the IC50 value of the drug) was used.

“Even at half the concentration, the toxicity of the approved drug miltefosine hydrate is higher than the tested inhibitor,” says Dr. Akhter. The screened molecule appears to have therapeutic efficacy with lower toxicity compared with miltefosine hydrate.

Though the protein sequence of *Leishmania* major was used, the in vitro studies using the screened molecule were carried out on *Leishmania donovani.*

“The UGM of *L. major* and the UGM of *L. donovani* have highly similar sequences. All the active regions are 100% identical. Hence these two can replace each other and a molecule that acts as an inhibitor for one protein will also act as inhibitor for the other. As the parasite strain available in the laboratory was *L. donovani*, the cell-based assays were performed on that,” says Dr. Akhter.

A study of nearly 300 people living in different parts of India found that nine single-base variants (single-nucleotide polymorphisms or SNPs) account

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