The resistance roadblock

For over two decades, Dr Rita Mukhopadhyay has been fascinated by the deadly parasite responsible for causing leishmaniasis. Here, she discusses how her extensive research is paving the way for the identification of promising new drug targets.

How did your interest in the drug resistance of Leishmania develop? In what ways has your research focus evolved over the course of your career?

As a Master’s student, I regularly volunteered at the Calcutta School of Tropical Medicine, India, and it was there that I saw several patients suffering from visceral leishmaniasis. A few were not responding to the treatment, which intrigued me and motivated me to start reading about it. I had already participated in classes on parasitology so I knew about the causative agent, *Leishmania donovani*. Further down the line, when I was in the graduate school at Jawaharlal Nehru University, New Delhi, I changed labs – and the opportunity to work with this parasite practically landed in my lap. I fell in love with parasitic research and today our 25-year love affair is still going strong. The way that this parasite always manages to stay one step ahead of our efforts to control it never ceases to amaze me.

**Can you explain the mode of action of the existing antimonial treatments – Pentostam and Glucantime?**

Several mechanisms have been proposed regarding the mode of action of antimonials. However, in my opinion, the key factor is the reduction of pentavalent antimonials through a reductase in macrophages and the subsequent transport of the trivalent form through *Leishmania* aquaglyceroporin (AQP1) – the only trivalent antimony facilitator in *Leishmania*. Since trivalent antimony reacts very strongly with thiols, it kills the parasite by depleting free thiols and deactivating key enzymes with cysteines in their active sites. The parasite depends on thiols such as trypanothione in order to combat oxidative stress while living inside the phagolysosome of the host’s macrophage.

**Can you summarise the evidence that has led you to investigate the potential of aquaglyceroporin channels as a novel drug target to address this resistance?**

Back in 2003, my group was investigating human AQP’s as trivalent arsenic channels.

A growing threat

Researchers at Florida International University, USA, are investigating the molecular mechanisms behind the alarming increasing virulence and resistance of *Leishmania* parasites.

**EVERY YEAR ABOUT** 12 million people are infected with a protozoa parasite spread by biting sand flies. Known as leishmaniasis, the resulting disease is endemic in areas of 88 countries across five continents, with the greatest burden found in the tropics and subtropics. Associated with malnutrition, poor housing and a weak immune system, it primarily afflicts the poorest people in the world and is responsible for an annual death toll of between 20,000 and 30,000. The disease exists in three main forms: visceral, cutaneous and mucocutaneous leishmaniasis.

Worryingly, over the course of the past decade a sizeable increase in the global incidence of leishmaniasis has been reported, as well as a wider geographical spread. These patterns have been linked to a combination of environmental and socioeconomic factors – such as deforestation, urbanisation, conflict and the collapse of public health infrastructure – and the emergence of HIV/leishmaniasis comorbidity. Unfortunately, the growing incidence of this disease coincides with its proliferating drug resistance. At present, pentavalent antimonials – namely, Pentostam and Glucantime – are the first line of defence against leishmaniasis-causing parasites – yet, in northern India alone over 50 per cent of visceral leishmaniasis cases are resistant to Pentostam. Antimonial resistance is thought to be a consequence of patient noncompliance, which facilitates the slow, underlying exposure of the parasite to the drugs.

One researcher who is taking the threat posed by leishmaniasis very seriously is Dr Rita Mukhopadhyay, Associate Professor in the Department of Cellular Biology and Pharmacology at Florida International University. Having devoted the past 25 years of her research career to studying *Leishmania* parasites, one of her main research objectives is to forge a deeper understanding of the molecular mechanisms that underpin their growing drug resistance. “The *Leishmania* genome is highly plastic and constantly employs novel mechanisms to survive in hostile environments,” she elucidates. “Pinpointing these mechanisms is extremely challenging, especially as *Leishmania* also displays strain-specific variability when responding to antimonial drugs – yet it is essential if we are to combat clinical resistance.”

Mukhopadhyay and her colleagues have focused on mapping the mechanisms of drug action and resistance in the treatment of leishmaniasis.

**STUDIES IN RESISTANCE**

In their research, Mukhopadhyay and her colleagues have focused on mapping the mechanisms of drug action and resistance in the treatment of leishmaniasis. Supported by the findings of other researchers, they
in collaboration with Professors Peter Agre and Barry Rosen. At this time, we also discovered modulation of arsenic sensitivity in leukaemia cells by human AQP9. Since nothing was known about antimony uptake systems in Leishmania, I was intrigued by the fact that arsenic and antimony are related metalloids and therefore there must be an AQP responsible for their uptake. Thus, my group identified the first AQP in *Leishmania* and showed its involvement in antimonial resistance. Several groups have since corroborated our findings with the filed isolates. Since AQP1 is essential for the parasite’s survival inside the macrophage, as well as during transmission, our rational conclusion was that it could be used as a drug target.

**Are there any other mechanisms of resistance that you are investigating as potential drug targets?**

Dr Goutam Mandal, a member of my research faculty, and Srotoswati Mandal – my research assistant – are currently working to identify the RNA interactome of AQP1. At the same time, my graduate student Mansi Sharma is investigating the post-translational regulation of AQP1 through the use of global interactome methodologies. From this research, we expect to identify novel targets that could also be used as drug targets.

**What progress has your research made towards finding a treatment to overcome this resistance? Have you identified any other promising drug candidates?**

We are on the tip of the iceberg in our research and still have a long way to go. At present, we are exploring the possibility that unique regulators of AQP1 could be reasonable targets. For example, since protozoan MAP kinases are distantly related to human MAP kinases, it might be possible to design compounds that specifically target the protozoan enzymes. We previously showed that *Leishmania* MAPK2 stabilised AQP1 by phosphorylation. Thus, one approach could be the development of specific small molecule inhibitors of *Leishmania* MPK2 activity. Such compounds would prevent the phosphorylation of the protozoan AQP1 channel, resulting in its increased turnover. We predict that these parasites would be killed by osmotic stress. A second approach could involve identifying small molecule inducers of MPK2, which may stabilise AQP1 and either reverse drug resistance or lower drug toxicity by reducing the effective dose.

**INTELLIGENCE**

**A NOVEL AQUAPORIN FROM *LEISHMANIA*: ROLES IN PHYSIOLOGY AND ANTIMONIAL RESISTANCE**

**OBJECTIVES**

To investigate the underlying mechanisms causing increased antimonial resistance in *Leishmania* parasites and identify novel therapeutic targets and drugs that could improve the effectiveness of treatment for leishmaniasis.

**COLLABORATORS**

- **Professor Barry P Rosen**, Professor Hiranmoy Bhattacharjee, Florida International University, USA
- **Professor Peter Agre**, Johns Hopkins Malana Research Institute, USA
- **Professor Scott Landfar**, Professor Larry David, Oregon Health and Science University, USA
- **Professor Marc Ouellette**, Canadian Institutes of Health Research, Canada
- **Professor Barbara Papadopoulou**, Université Laval, Canada
- **Professor Dr Eric Beitz**, University of Kiel, Germany
- **Professor Markus Tamas**, University of Gothenburg, Sweden
- **Professor Dr Ritala Mukhopadhyay**, Herbert Wertheim College of Medicine

**FUNDING**

National Institutes of Health (NIH)
Florida International University – Herbert Wertheim College of Medicine

**CONTACT**

Dr Ritala Mukhopadhyay
Associate Professor
Cellular Biology and Pharmacology
Herbert Wertheim College of Medicine
Florida International University
Miami 33199
Florida
USA
T +1 305 348 1472
E rmukhop@fiu.edu, rmukhop@gmail.com

**RITA MUKHOPADHYAY** gained her PhD from Jawaharlal Nehru University, India, in Parasitology and Biochemistry before moving to the US for a postdoctoral position at Wayne State University. In 2008, she moved to Florida International University. Mukhopadhyay has authored 43 peer-reviewed articles and 10 book chapters and, in addition to her research, teaches passionately at the undergraduate and postgraduate levels and supervises postgraduate research.

The Mukhopadhyay Group

**The Mukhopadhyay Group**

Dr Ritala Mukhopadhyay’s most recent publications can be viewed here: [http://bit.ly/RM_publications](http://bit.ly/RM_publications)