Rational designs

As an expert in medical microbiology with many years of experience in the field, Professor Dr Erik C Böttger is ideally placed to help guide the development of rationally designed drugs. Here he expounds upon the wider implications of his work.

Another aminoglycoside, paromomycin, is also effective in targeting parasitic infections by protozoa such as Leishmania. What is the antiprotozoal mechanism of this drug and why did you then choose to extend your studies to investigate Trypanosoma?

Paromomycin is an established agent for treatment of leishmaniasis. However, for many years its mechanism of antiprotozoal action has remained elusive. Using genetic reconstructions of the protozoal drug binding sites in bacteria we concluded that paromomycin acts by targeting protozoal cytosolic protein synthesis. Molecular analysis revealed that the drug binding pocket of Trypanosoma resembles that of Leishmania, leading to the hypothesis that paromomycin should also be active towards Trypanosoma parasites. The antityranosomal activity of paromomycin was subsequently established in *in vitro* cell culture assays and *in vivo* in mice infection models.

Do you envisage your work as leading to the development of new treatments for these devastating parasitic infections? What needs to be done to see this research being translated into novel therapeutics?

I think our work exemplifies and demonstrates the enormous potential of the ribosome as a drug target for many of the poverty-related infectious diseases, including parasite infections. By establishing proof of principle for a mechanistic hypothesis addressing both the issue of drug specificity and toxicity, we have provided paradigmatic and exemplary showcases that demonstrate the many avenues offered by ribosomes as targets for new treatments.

In general, the main problem in contemporary drug development is not activity, but toxicity. As far as the ribosome as a drug target is concerned, tools are available to rapidly characterise a compound for selectivity and on-target mechanism of action-related toxicity. Clearly, the road to novel therapies is a long one and can only be reached with support and commitment both from public grant agencies and the pharmaceutical industry.

Your achievements in the field of microbiology are extensive, what has been the key to maintaining such varied and innovative research projects?

There are many different approaches to scientific achievements; as many as there are scientists. Personally, I have found that the key to innovation is focusing on a different aspect of my research every 10 years or so. This has helped me to combine experience with the learning attitude and curiosity of a student.

How do you see your research progressing in the future? What do you currently have in the pipeline?

The increasing incidence of antibiotic resistance and the toxicity associated with some of the available agents constitutes a formidable challenge for further exploitation of the ribosome as a drug target. A major part of our drug discovery programme will continue to focus on aminoglycoside antibiotics, a class of compounds categorised by the World Health Organization (WHO) as one of the most critically important antimicrobials for human therapy. Based on the mechanistic hypothesis discussed above; backed up by our findings with apramycin; and driven by iterative rounds of chemical synthesis and selectivity testing, we have recently identified a new aminoglycoside chemophore with increased target selectivity towards bacterial ribosomes, and little activity for any of the human ribosomal drug-binding pockets (*Nature Communications*, in press).

While our focus is on aminoglycosides we also have a keen interest in other ribosomal antibiotics. For example, we have teamed up with Professor Richard E Lee from St Jude Children’s Research Hospital in a National Institute of Health (NIH)-funded international consortium to successfully transform spectinomycin into an antituberculosis compound (*Nature Medicine*, in press).
Resisting antibiotic resistance

Antibiotics were one of the most important medical breakthroughs of the last century. The medical research community can never be complacent however, as they are fighting against highly adaptable organisms. Fortunately, work is underway at the University of Zurich to develop improved antibiotics.

ALEXANDER FLEMING’S OBSERVATION in 1928 that penicillin had antibacterial properties sparked the beginning of a revolution in healthcare. Suddenly, deadly diseases such as bacterial meningitis became treatable, along with TB, whooping cough and pneumonia. Antibiotics have saved millions of lives, doctors now have dozens available as treatment options and hundreds of millions of people are prescribed them every year.

However, Fleming’s legacy is beginning to fade. Scarce 50 years after their widespread introduction, there are serious concerns associated with the future of these lifesaving drugs. Bacterial populations develop resistance to antibiotics, allowing them to slip through the net of treatment. The overuse of these treatments worldwide – especially in developing countries where many are available over the counter – is rapidly accelerating us towards a world in which antibiotics are entirely ineffectual. New forms of highly resistant bacteria have emerged, and every year 25,000 patients in the EU alone are killed by such strains. Unfortunately, even with modern industrial drug discovery methods, no new classes of antibiotics have been found in over 20 years.

Despite this seemingly bleak situation, some researchers are producing encouraging work. Professor Dr Erik C Böttger from the University of Zurich’s Institute of Medical Microbiology is part of an international team of researchers that has been working to significantly improve one of the most powerful classes of antibiotics in modern medicine’s arsenal. Aminoglycoside antibiotics show potential to treat even the most evasive bacteria, but they are hampered by many serious side-effects. This means the gap between a toxic dose and a therapeutic dose is incredibly narrow. Aminoglycosides can seriously damage hearing, with 20 per cent of patients who take them on a short course experiencing partial hearing loss. Despite these side-effects, aminoglycosides are some of the most widely prescribed antibiotics due to their high efficacy and low cost.

DESIGNING DRUGS RATIONALLY

One of the difficulties in modern medicine is implementing effective drug delivery. In order to get the most therapeutic effect with the least harm, antibiotics should only target pathogenic invaders, while leaving human cells untouched. Unfortunately, a cellular remnant of our evolutionary heritage can make this difficult. The organelles that generate ATP – our molecular fuel – are called mitochondria, and within the mitochondria lie ribosomes, which can be thought of as the machines that build proteins. The story of early cell evolution is a patchy one; but the incredible similarity that mitochondrial ribosomes bear to those found in bacteria lends support to an intriguing theory of early cell development. It is thought that originally mitochondria were actually separate living entities, living in a symbiotic relationship with precursor animal cells, before becoming incorporated into the cell entirely.
INTELLIGENCE
GENETIC RECONSTRUCTION OF PROTOZOA rRNA DECODING SITES PROVIDES A RATIONALE FOR PAROMOMYCIN ACTIVITY AGAINST LEISHMANIA AND Trypanosoma

OBJECTIVES

• Developing novel, rationally-designed drugs to target bacterial ribosomes while avoiding damage to human cells

• Investigating the antiparasitic activity of paromomycin, an aminoglycoside which has proved useful in combating Leishmania and now Trypanosoma protozoan parasites

KEY COLLABORATORS

Professor Dr Andrea Vasella, Laboratory of Organic Chemistry, ETH Zurich, Zurich, Switzerland • Professor Dr Venki Ramakrishnan, Structural Studies Division, MRC Laboratory of Molecular Biology, Cambridge, UK • Professor Dr Jochen Schacht, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, Michigan, USA • Professor Dr David Crich, Department of Chemistry, Wayne State University, Detroit, Michigan, USA • Professor Dr Richard E Lee, Department of Chemical Biology and Therapeutics, St Jude Children’s Research Hospital, Memphis, Tennessee, USA

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PROFESSOR DR ERIC C BÖTTGER

is internationally recognised for his contributions to the field of antibiotic resistance, bacterial genetics and drug development. His activities have resulted in over 200 original research publications, 15 book contributions and various review articles. He has received numerous awards, including the prestigious Körber Award for European Science, and he is ranked among the world’s most cited and influential microbiologists of the past two decades.

Leishmania and Trypanosoma

Two genera of trypanosomatid, parasitic protozoa which are spread by insect vectors and are both responsible for an assortment of life-threatening diseases:

Leishmaniasis – spread by sandflies and affecting around 12 million people worldwide, can cause disfiguring sores to appear on the skin and can be fatal in its cutaneous or visceral forms.

Trypanosomiasis – a name given to a collection of deadly diseases that includes sleeping sickness and Chagas disease (African and American trypanosomiasis, respectively). The parasites are spread by tsetse flies and kissing bugs, and cause an array of alarming symptoms and often death.

This evolutionary accident was a great boon to the expansion of early life, but means that antibiotics targeting the ribosomes of disease bacteria can often damage host cells as well.

Böttger’s team postulated that the damage to the outer hair cells within the inner ear (ototoxicity) caused by aminoglycosides is the result of this similarity. These cells are what convert sound to neural impulses, and once they are damaged, they cannot grow.

The group followed this hypothesis and identified apramycin as a drug which acts on bacterial ribosomes but has little effect on mitochondrial ribosomes. If their idea as to why the aminoglycosides are ototoxic is correct, this drug should be non-toxic whilst retaining antibacterial properties.

APRAMYCIN AND PAROMOMYCIN

Apramycin is a structurally unique aminoglycoside that hardly acts on human mitochondrial ribosomes. Currently licensed for use by veterinarians, the team has set the stage for human trials. Using both human cochlear cell cultures and guinea pig trials, they have demonstrated that apramycin indeed causes very little damage to the outer hair cells of the inner ear. Furthermore, they showed that apramycin has excellent antibacterial properties, attacking a range of resistant pathogens including multidrug-resistant TB.

Aminoglycosides, specifically paromomycin, also provide excellent treatment for the parasitic infection leishmaniasis. Having identified the molecular mechanisms by which this drug targets the protozoan parasites in question, the team inferred that it may also be effective in combating infection by Trypanosoma parasites, which have very similar drug binding pockets.

A NEW MODEL FOR MEDICAL RESEARCH

More broadly, this work is exciting because it shows the potential of rational drug design. Researchers had previously attempted to synthesise less toxic aminoglycosides by trial- and-error, but having identified the mechanism behind the negative side-effects, Böttger’s team was able to identify a promising compound very quickly.

By creating hybrid bacteria with the relevant human ribosome binding sites, it has been possible to easily guide the synthesis of drugs that affect the bacterial ribosome more strongly than the human ribosome. This is a major step forward. Examining the activity of the drug against the ribosome can effectively identify drugs that target the intended site. By placing the relevant sites into the bacterial cell, it is possible to simultaneously screen drugs for their overall antibacterial activity and their action against specific sites. A current trend in medical science is a drive towards more rationally designed drugs, in an effort to avoid the incredibly high cost of current drug development, which has been estimated at US $1 billion for every new molecular entity discovered.

The fight against bacterial resistance to antibiotics will have to be a multidisciplinary one. Healthcare practitioners need to ensure that only those that are truly in need get prescribed antibiotics, and politicians need to establish regulatory mechanisms in developing countries to restrict the flow of over-the-counter antibiotics. On the research side, the drug discovery paradigm has been falling when it comes to antibiotics, and will soon reach a stage when we are desperate for novel drugs to combat incredibly resistant future strains of bacteria. The massive amount of work being undertaken to understand cellular processes, combined with innovative drug selection strategies like the one employed by Böttger’s team here, will hopefully bring a second golden age of antibiotics to fruition. The stakes in medical research have never been higher.