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INFECTIOUS DISEASES

Decrypting *Cryptosporidium*

Infections that cause diarrhoea — such as cryptosporidiosis, caused by the protozoan parasites *Cryptosporidium hominis* and *Cryptosporidium parvum* — are responsible for nearly a million deaths every year, mostly among children in low-income countries as well as immunodeficient patients. Unfortunately, drug development against *Cryptosporidium* spp. has been hampered by technical challenges. However, new results by Manjunatha *et al.*, who have identified *Cryptosporidium* phosphoinositide 4-kinase (CpPI4K) as a therapeutic target and the pyrazolopyridine KDU731 as a potent inhibitor of cryptosporidiosis, are hopeful.

To discover potential agents against cryptosporidiosis, the authors screened 6,220 compounds with known activity against several protozoan parasites and identified 154 compounds that inhibited *C. parvum* growth by more than 60%. In a subset of pyrazolopyridine analogues, the authors found that activity against *C. hominis* (which is responsible for most clinical infections) and *C. parvum* correlated with activity against *Plasmodium falciparum*, which suggested that the mechanism of action of these pyrazolopyridines is conserved between these two parasites.

> “These results reveal CpPI4K as an important target in the treatment of cryptosporidiosis as well as the potential therapeutic value of KDU731.”

Pyrazolopyridines are known to exert their antimalarial activity through inhibition of the *Plasmodium* spp. lipid kinase PI4K. Therefore, the authors searched for potential *Cryptosporidium* spp. orthologues of this kinase and found that the PI4K homologues in *C. hominis* and *C. parvum* (CpPI4K) show remarkable amino acid sequence similarity to *P. falciparum* PI4K. Among the screened compounds, the imidazopyrazine KDU691 and the pyrazolopyridine KDU731 were shown to be potent inhibitors of CpPI4K enzymatic activity — by binding to the ATP-binding site of the enzyme — with the most favourable selectivity window over human PI4K.

A drug to treat cryptosporidiosis should be suitable for use in children under the age of 2 years who are likely to be receiving other treatments for comorbidities, so safety and minimal risk of drug–drug interactions are key requirements. With that in mind, out of the two inhibitors, the authors chose KDU731 for further validation, as it showed a safe profile in a battery of safety pharmacology assays and did not inhibit any of the major forms of cytochrome P450.

To test the efficacy of the compound *in vivo*, the authors inoculated interferon-γ (IFNγ)-deficient mice — which are highly susceptible to infection with *C. parvum* — with 10,000 transgenic parasites expressing nanoluciferase, which enabled monitoring of infection levels by measuring luminescence in the faeces. Orally administrated KDU731 greatly reduced faecal oocyst shedding, and no parasites were observed in the intestines of KDU731-treated animals after the course of the treatment, whereas those that received vehicle treatment showed substantial infection levels.

Efficacy was also tested in newborn calves, which are naturally susceptible to *C. parvum* infection and display a pathophysiology that closely matches that observed in children. Calves received 5 mg per kg of oral KDU731 2–4 days after infection, twice a day for 1 week. The treatment was well tolerated, and resolution of symptoms started as early as 24 h after treatment. Within 3 days of treatment, calves shed significantly fewer oocysts and suffered severe diarrhoea for fewer days than calves that received vehicle treatment.

These results reveal CpPI4K as an important target in the treatment of cryptosporidiosis as well as the potential therapeutic value of KDU731. According to the authors, the synthetic route and formulation envisaged are relatively simple to enable a low final cost, taking into account the need in low-income countries; however, no formal accurate projections of cost can be made at this stage of the project. Further safety and pharmacological preclinical evaluation of this compound to support the initiation of clinical trials in patients is eagerly awaited.

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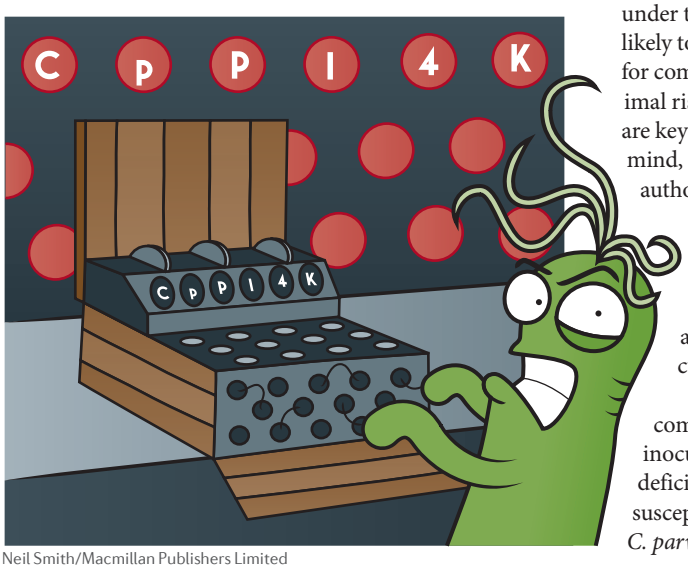

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