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ANTIPARASITIC DRUGS

Proteasome inhibition combats kinetoplastid infections

Chagas disease, leishmaniasis and sleeping sickness are caused by infection with kinetoplastid parasites and are prevalent among the world's poorest populations. Although treatment options for these diseases exist, their use is limited by several factors, including toxicity, poor efficacy, difficult administration and cost. Now, writing in *Nature*, Supek and colleagues identify a selective inhibitor of the kinetoplastid proteasome that effectively clears infection in mouse disease models.

Kinetoplastid parasites exhibit similar biology and genomic sequence, suggesting that associated diseases could all be treated by a drug that modulates the activity of a common parasite target. To identify such an agent, Supek and colleagues first screened more than 3 million compounds for their ability to inhibit growth of each of the parasites *Trypanosoma cruzi* (*T. cruzi*), *Leishmania donovani* (*L. donovani*), and *Trypanosoma brucei* (*T. brucei*), which cause Chagas disease, leishmaniasis and sleeping sickness, respectively.

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This screen led to the identification of an azabenzoxazole, GNF5343, as a particularly potent inhibitor of kinetoplastid parasite growth. Optimization generated GNF6702, which had reduced risk of toxicity, improved selectivity over mammalian cell growth inhibition, low clearance, acceptable bioavailability and a 400-fold increase in potency against intramacrophage *L. donovani* compared with GNF5343.

The efficacy of GNF6702 was then tested in four mouse models of kinetoplastid infection. In mice infected with *L. donovani* (which causes the majority of visceral leishmaniasis infections), twice daily oral dosing with GNF6702 for 8 days reduced liver parasite burden by up to more than 99%, which was greater than that achieved with miltefosine, the only clinically available oral anti-leishmanial drug. Twice daily oral GNF6702 treatment was similarly more effective than miltefosine in a mouse model of cutaneous leishmaniasis: it significantly decreased footpad parasite burden and swelling in mice with footpad *Leishmania major* infection.

In addition, twice daily oral treatment of mice with GNF6702 beginning 35 days after *T. cruzi* infection matched the efficacy of orally administered benznidazole, an agent broadly used to treat acute and indeterminate stages of Chagas disease but which exhibits significant side effects. Parasites were undetectable in the blood, colon and heart of all but one GNF6702-treated mice, even after 4 weeks of immunosuppression. Furthermore, in mice with established *T. brucei* infection in the central nervous system (which mimics stage II sleeping sickness), once daily oral treatment with GNF6702 for 7 days eliminated detectable parasites in the brain, whereas parasites were detected in all mice treated intraperitoneally with diminazene aceturate, a stage I drug that minimally crosses the blood–brain barrier.

Evolution of parasite strains resistant to GNF6702 or its analogues indicated that the parasite proteasome was the primary pan-kinetoplastid drug target of GNF6702. Specifically, an F24L mutation in the proteasome β4 subunit conferred selective resistance of *T. cruzi* epimastigote isolates and *T. brucei* bloodstream trypomastigotes to the growth inhibitory effects of GNF6702. Biochemical assays revealed that GNF6702 blocked kinetoplastid parasite growth by inhibiting proteasome chymotrypsin-like activity. Importantly, GNF6702 had no measurable activity on the human proteasome.

In summary, these findings validate the parasite proteasome as a promising therapeutic target for the broad treatment of kinetoplastid infections. GNF6702 is currently being evaluated in preclinical toxicity studies.

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