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Short Communication

Demonstration of the Anthelmintic Potency of Marimastat in the Heligmosomoides Polygyrus Rodent Model

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Abstract: In the course of a structure-based drug discovery program the known anticancer candidate marimastat was uncovered as a potent inhibitor of an enzyme in nematode cuticle biogenesis. It was shown to kill Caenorhabditis elegans, and the sheep parasites Haemonchus contortus and Teladorsagia circumcinta via an entirely novel nematode-specific pathway, specifically by inhibiting cuticle-remodelling enzymes that the parasites require for the developmentally essential molting process. This discovery prompted an investigation of the
compound’s effect on *Heligmosomoides polygyrus* parasites in a mouse model of helminth infection. Mice were administered the drug via oral gavage daily from day of infection for a period of 2 wk. A second group received the drug via intra-peritoneal implantation of an osmotic minipump for 4 wk. Control groups were administered identical volumes of water by oral gavage in both cases. Counts of *H. polygyrus* fecal egg and larval load showed that marimastat effected a consistent and significant reduction in egg laying, and a consistent but minor reduction in adult worm load when administered every day, starting on the first day of infection. However, the drug failed to have any significant effect on egg counts or worm burdens when administered to mice with established infections. Therefore, marimastat does not appear to show promise as an anthelmintic in gastrointestinal nematode infections, although other metalloproteases such as batimastat may prove more effective.

Pathogenic nematodes are the cause of significant levels of disease in man and livestock. Over one billion people are infected, the majority living in the developing world, causing a heavy medical and economic burden (Hotez et al., 2008; Geary, 2012). The gastrointestinal parasitic nematodes of the trichostrongylidae family infect grazing livestock and are found worldwide. They cause emaciation, anemia, and even death of the host animal, resulting in a significant impact on animal welfare as well as economic consequences for the farmer (Grencis, 2015). *Haemonchus contortus* is a very common parasite, and in terms of the global agriculture industry, one of the most pathogenic. Haemonchosis results in large losses for farmers, particularly those living in warmer climates (Gilleard, 2013). The incidence and cost of the disease are growing, with the parasite now being found in countries previously free of the disease, including the U.K. It is thought that a combination of livestock transportation, climate change and resistance to the anthelmintic drugs used to treat the condition is the cause of the spread (Kaplan, 2004; Wolstenholme et al., 2004).
Reports of anthelmintic resistance to multiple drugs in individual parasite species, and in multiple parasite species across virtually all livestock hosts, are common and growing (Kaplan and Vidyashankar, 2012). Instances of nematode resistance to 3 different anthelmintics have now also been documented (Papadopoulos, et al., 2012). The ability of the parasites to survive treatments that are normally effective at the recommended dose rate is considered a major threat to the future control of the disease (Shalaby, 2013). There are very few vaccines available for gastro-intestinal nematode prevention and so new compounds that affect novel nematode drug targets are urgently required. This is not trivial, as evidenced by the fact that in the last 25 yr, only 2 new classes of anti-nematode drugs have been found: the cyclodepsipeptides (Lemmens-Gruber et al., 2009) and the aminoacetonitriles (Kaminsky et al., 2008).

In nematodes, the cuticle is a collagenous extracellular matrix (ECM) synthesized by an underlying ectodermal cell layer called the hypodermis that surrounds the body of the animal. Enzymatic digestion of the cuticle occurs during molting via matrix metalloproteases, where the cuticle is softened and then shed, ready for the newly synthesized layer (Singh and Sulston, 1978). The cuticle and the molting process in *C. elegans*, in particular, have been examined most thoroughly (Cox et al., 1981; Johnstone, 1994; Kramer, 1994), and it has been shown that chemical and genetic inhibition of the nematode cuticle moulting process results in death of the organism (Page et al., 2014). This molting process involves a specific class of well-characterized astacin metalloproteases, including the procollagen C-proteinase DPY-31. Recently, a combined ligand- and structure-based inhibitor approach identified a range of metalloprotease inhibitors that inhibit DPY-31 in vitro from both the human filarial nematode *Brugia malayi*, and the parasitic gastrointestinal nematode of sheep *Teladorsagia circumcincta*. In vivo these inhibitors also elicit the severe body morphology defect ‘Dumpy’ (Dpy; shorter and fatter), a predominantly non-viable phenotype consistent with mutants.
lacking the DPY-31 gene (France et al., 2015). These types of inhibitor also often induce molting defects, which are often fatal to the organism (Stepek et al., 2015). One of the most potent of these compounds was marimastat, a broad-spectrum matrix metalloprotease inhibitor previously studied as an antineoplastic in clinical trials (Vandenbroucke and Libert, 2014; Cathcart et al., 2015).

The nematode *H. polygyrus* is a natural parasite of rodents (Maizels et al., 2012). It pursues a direct and entirely enteric life cycle, similar to trichostrongylid nematode of veterinary importance, entering through the mouth and maturing in the intestine to produce eggs which are voided with feces. It is a valuable laboratory model as it can establish chronic infection in different strains of mice (Johnston et al., 2015). The aim of this proof-of-concept study was to examine the effect of marimastat on GI parasite infection to determine whether the effects noted in vitro on free-living, filarial and trichostrongylid nematodes would translate to an in vivo *H. polygyrus* model of the disease.

*Heligmosomoides polygyrus* infection. C57BL/6 female mice, aged 6-8 wk, were infected by oral gavage of 200 *H. polygyrus* L3 in 200 μl water. The *H. polygyrus* lifecycle was maintained and the L3 larvae obtained as previously described in (Johnston et al., 2015).

**Oral drug administration:** Mice were given marimastat (1 mg/200 μl water) by gavage daily from the first day of infection (day 0) for 2 wk. On the day of infection, marimastat gavage was given 4-6 hr following parasite infection. Control mice received a gavage containing 200 μl water. Mice then received daily gavages of marimastat or water as appropriate from days 1-8. At day 14 egg and worm counts were performed.

**Minipump administration:** Administration of marimastat was effected by intraperitoneal implantation of an osmotic minipump (ALZET 1004, DURECT Corporation, Cupertino, CA) which released 110 nl per hour for 4 wk. Minipump implants contained either
marimastat (42 mg/100 μl 50% DMSO) or vehicle alone (100 μl 50% DMSO). At days 14 and 21 egg counts were performed followed by worm and egg counts on day 28.

Administration of drug to established adult worm infections: Mice infected with 200

*H. polygyrus* L3 in 200 μl water via oral gavage at day 0, received daily gavages of marimastat (1 mg/200 μl water) or 200 μl water from days 9-17 of infection. At day 14 and day 21 egg counts were performed followed by worm and egg counts on day 28.

The egg burdens of individual mice were assessed by collecting 2-3 fecal pellets for each *H. polygyrus*-infected mouse at the specified time intervals. Feces were weighed before being dissolved in 2 ml PBS followed by the addition of 2 ml saturated sodium chloride solution. Egg counts were then carried out with the use of a McMaster chamber and the average number of eggs/g feces calculated per sample. At the end point of each study, the intestinal adult worms were also counted to give the total worm burden of each individual mouse; the small intestinal tissues were recovered and total worm burdens enumerated with the aid of a dissecting microscope

Effects of oral marimastat administration: Mice infected with *H. polygyrus* were exposed to marimastat by oral gavage, and levels of infection measured. After 14 days daily gavage of marimastat solution, a reduction of almost 50% in eggs was observed (Fig. 1A) when compared to the control group gavaged with water alone. However, due to the small sample size and large variability in the control group, this difference achieved only marginal statistical significance (p = 0.053). At the same time point, total intestinal worm counts were performed, however, no effect of marimastat was evident in this measure (Fig. 1B).

Effects of intraperitoneal administration of marimastat: A second route of drug administration was then tested, by intraperitoneal implantation of an osmotic minipump that continuously released marimastat over a 4 wk period. Although no effect was evident at day 14 (Fig. 2A)
modest reductions in egg counts in the marimastat minipump group, compared to control mice implanted with vehicle-alone minipumps, were observed at both day 21 and day 28 (Fig. 2B, C). These differences were consistent (reducing egg counts by 28% at each time point) albeit not statistically significant (p = 0.229). In contrast to the outcome of oral administration, some worm killing effect was also suggested with a 30% reduction, although not reaching statistical significance (p = 0.312), (Fig. 2D).

Treatment of established adult worm infections: Finally, the effects of marimastat on an established H. polygyrus population was assessed by oral administration from day 8 of infection, by which time adult worms are established in the intestinal lumen. Although not statistically significant, a 40.8% reduction in egg counts was observed (p = 0.170; Fig. 3A). However, no reduction in adult worm numbers was found (Fig. 3B), indicating that established adult worm infections may be more resistant to marimastat than nascent ones.

All of the egg counts from the final time points of all 3 experiments (n = 15) were aggregated to perform a final t-test between the marimastat group and the control group, which established a statistically significant reduction in egg counts (p = 0.048), with an effect size of 0.76 (where by convention an effect size of 0.5 is considered medium and 0.8 large (Sawilowsky, 2009)).

Aggregating the results from all 3 experiments reveals a consistent and statistically significant reduction in egg counts but an inconsistent and negligible reduction in adult worm load when the drug marimastat is given to mice during the period of larval invasion and establishment. Furthermore, although adult worm killing by marimastat is not evident, the reduction in egg counts suggests that those adult nematodes not killed by the drug are still experiencing deleterious effects from it.
Heligmosomoides polygyrus is a murine nematode related to the highly prevalent trichostrongylids of livestock and is a tractable model for screening novel drug compounds. In our studies, we noted that both the gavage process and minipump implantation (both widely used delivery methods in clinical research) increased the susceptibility of mice to infection, compared to animals experiencing no physical intervention. Stress is well known to impair immune responses (Joana et al., 2016; Levi et al., 2016), and this confounding effect may also have reduced the observed potency of the compound.

We have previously reported that marimastat produces deleterious effects on pathogenic nematodes in vitro (France et al., 2015), and here it shows significant effects on nematode egg production in an animal model when administered orally from the first day of infection. However, the drug failed to have any significant effect on egg counts or worm burdens when administered to mice with established infections, or when administered via osmotic minipump. Therefore, marimastat does not appear to show promise as an anthelmintic in gastrointestinal nematode infections. Marimastat is known to be rapidly metabolized in rodents which may have contributed to the weak worm killing power observed (Rasmussen and McCann, 1997). The related compound batimastat is known to be less rapidly metabolized and may show more potency in this particular type of assay; this and other related compounds previously developed to enhance plasma stability (Hermant et al., 2017) should be investigated.

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LITERATURE CITED


Figure 1. Effects of oral marimastat administration. Mice infected with *Heligmosomoides polygyrus* were exposed to marimastat by oral gavage daily for 2 wk, and levels of infection measured (A) egg count; (B) worm count. Control mice received a gavage containing 200 μl water.

Figure 2 Effects of intraperitoneal administration of marimastat. An osmotic minipump released either marimastat or vehicle alone for 4 wk. At days 14 (A) and 21 (B) egg counts were performed followed by egg and worm counts on day 28 (C, D, respectively).

Figure 3 Treatment of established adult worm infections. Mice infected with *Heligmosomoides polygyrus* L3 via oral gavage at day 0 received daily gavages of marimastat or water from days 9-17 of infection. Results are shown for the worm (A) and egg (B) counts performed on day 28.